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RESEARCH PAPER

Involvement of Organic Cation Transporters in the Clearance and Milk Secretion of Thiamine in Mice

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ABSTRACT

Purpose To investigate the role of organic cation transporters (Octs) and multidrug and toxin extrusion protein 1 (Mate1) in the disposition of thiamine.

Methods The uptake of [^{3}H]thiamine was determined in Oct1-, Oct2-, and Oct3-expressing HEK293 cells and freshly isolated hepatocytes. A pharmacokinetic study of thiamine-d_3 following intravenous infusion (1 and 100 nmol/min/kg) was conducted in male Oct1/2(+/+) and Oct1/2(−/−) mice. A MATE inhibitor, pyrimethamine, (5 mg/kg) was administered intravenously. The plasma and breast milk concentrations of thiamine were determined in female mice.

Results Thiamine is a substrate of Oct1 and Oct2, but not Oct3. Oct1/2 defect caused a significant reduction in the uptake of [^{3}H]thiamine by hepatocytes *in vitro*, and elevated the plasma thiamine concentration by 5.8-fold *in vivo*. The plasma clearance of thiamine-d_3 was significantly decreased in Oct1/2(−/−) mice. At the higher infusion rate of 100 nmol/min/kg thiamine-d_3, Oct1/2 defect or pyrimethamine-treatment caused a significant reduction in the renal clearance of thiamine-d_3. The total thiamine and thiamine-d_3 concentrations were moderately reduced in the intestine of Oct1/2(−/−) mice but were unchanged in the kidney, liver, or brain. The milk-to-plasma concentration ratio of thiamine was decreased by 28-fold in the Oct1/2(−/−) mice.

Conclusions Oct1 is possibly responsible for the plasma clearance of thiamine *via* tissue uptake and for milk secretion. Oct1/2 and Mate1 are involved in the renal tubular secretion of thiamine.

KEY WORDS multidrug and toxin extrusion protein · organic cation transporter · thiamine · vitamin b1

ABBREVIATIONS

AUC	Area under the plasma concentration –time curve
BBM	Brush border membrane
CL_{plasma}	Total body clearance with regard to the plasma concentration
CL_R	Renal clearance
GFR	Glomerular filtration rate
LC-MS/MS	Liquid chromatograph-tandem mass spectrometer
MATE	Multidrug and toxin extrusion protein
MEC	Mammary epithelial cells

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MPP^+	1-methyl-4-phenylpyridinium
OCT	Organic cation transporter
Oct1/2(+/+) mice	Oct1 and Oct2 gene wild-type mice
Oct1/2(−/−) mice	Oct1 and Oct2 gene-knockout mice
R_b	Blood-to-plasma concentration ratio
RFC	Reduced folate carrier
TEA	Tetraethylammonium
THTR	Thiamine transporter
TMP	Thiamine monophosphate
TPK	Thiamine pyrophosphokinase
TPP	Thiamine pyrophosphate
X_{urine}	Urinary excretion amount

INTRODUCTION

The organic cation transport system in the body is comprised of organic cation transporters (OCTs/*SLC22A*) and multidrug and toxin extrusion proteins (MATEs/*SLC47A*) that mediate the tissue uptake and subsequent efflux, respectively, of various endogenous and exogenous cationic compounds (1). The OCT family consists of three isoforms OCT1-3/*SLC22A1-3*. OCT1 and OCT2 mediate the hepatic and renal uptake of organic cations from the systemic blood, respectively in humans, whereas OCT3 is ubiquitously expressed in the body, and its role in the disposition of drugs has not been established (2). Oct1 is also expressed in rodent kidney, where it mediates the uptake of cationic drugs together with Oct2 (3). Human MATE1 (*SLC47A1*) and MATE2-K (*SLC47A2*), and rodent Mate1 are expressed on the brush border membrane (BBM) of the renal proximal tubules, and MATE1 and Mate1 are also expressed in the canalicular membrane of hepatocytes (4). MATEs are capable of mediating the efflux of various cationic drugs with an exchange of H^+ across the plasma membrane, thereby acting as an efflux transporter in the kidney and liver.

Using a metabolomic analysis, we recently identified thiamine, which is also known as vitamin B_1, to be a urinary endogenous compound that was significantly decreased by pyrimethamine, a potent inhibitor for MATEs (5). Thiamine was transported by both MATE1 and MATE2-K *in vitro* using cDNA transfected cells (6). We also found that thiamine is an *in vitro* substrate of OCT2 as well as MATEs using cDNA transfected cells (5). Thiamine is transformed by thiamine pyrophosphokinase (TPK) into thiamine pyrophosphate (TPP) (7), which acts as a coenzyme in several key reactions of cellular metabolism by transketolase in the pentose phosphate pathway, by three mitochondrial enzyme complexes consisting of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase in the tricarboxylic acid (TCA) cycle and branched chain α-ketoacid dehydrogenase in amino acid catabolism, and by peroxisomal 2-hydroxyphytanoyl-CoA lyase in α-oxidation of 3-branched fatty acids (8,9). Thiamine deficiency results in beriberi, lactic acidosis, Wernicke-Korsakoff syndrome, central and peripheral neurological impairment, and cardiovascular disease (10,11). Thornalley *et al.* has reported that type 1 and type 2 diabetic patients in the UK exhibited low plasma thiamine concentration (12), and high-dose thiamine therapy reduced urinary albumin excretion (UAE), a primary endpoint of microvascular complications in diabetes (13). An improvement in the plasma thiamine concentration might be effective for decreasing the risk of microvascular complications.

Two thiamine transporters, THTR-1/*SLC19A2* and THTR-2/*SLC19A3* have been cloned (14–16). THTR-1 is widely expressed with the highest expression level in the skeletal muscle followed by placenta, heart, liver, and kidney (14), whereas THTR-2 is expressed at the highest level in the duodenum (15). In addition, THTR-1 is presumably localized in erythrocytes (12), whereas THTR-2 is localized in the kidney and brain (17,18). THTR-1 is responsible for thiamine-responsive megaloblastic anemia, which is characterized by diabetes, deafness, and anemia (19,20). Thtr-1(−/−) mice exhibit a diminished high affinity uptake of thiamine by erythrocytes (21). THTR-2 is responsible for biotin-responsive basal ganglia disease, which is characterized by recurrent sub-acute encephalopathy (22). Thtr-2(−/−) mice exhibit reduced intestinal absorption (23). Rat hepatocytes show a saturable uptake of thiamine that is inhibited by quaternary ammonium compounds such as choline (24), which has an inhibitory potential toward OCT1 (1) but not THTR-1 (14), indicating the possibility of hepatic thiamine uptake *via* Oct1 rather than Thtr-1. Thiamine is readily filtered by renal glomeruli and then reabsorbed from the glomerular filtrate in normal conditions (17). We estimated that more than 90% of thiamine is reabsorbed from the urine (5). However, when excess of thiamine is ingested, it is extensively eliminated into the urine *via* tubular secretion and glomerular filtration (25). Therefore, we investigated the role of OCTs in thiamine disposition both at physiological and excess levels of thiamine. Thiamine is an indispensable nutrient for infants (26). Recently, we observed that Oct1 was induced during the lactation stage in mammary epithelial cells (MEC) and was involved in the transfer of Oct1 substrates into milk (27). Therefore, OCT1 may mediate the secretion of thiamine in milk from the mammary glands.

In the present study, we investigated the role of Octs in the disposition of thiamine in mice by conducting an *in vivo* kinetic analysis using Oct1/2(−/−) mice. The results obtained in this study provide insight into the mechanism involved in thiamine disposition and broaden our understanding of the role of OCTs in the body.

MATERIALS AND METHODS

Materials

Thiamine hydrochloride, thiamine monophosphate (TMP) chloride dihydrate, pyrimethamine, tetraethylammonium (TEA) and lyophilized acid phosphatase from potato were purchased from Sigma-Aldrich (St. Louis, MO). Deuterium-labeled thiamine (thiamine-d_3) hydrochloride and beclotiamine hydrochloride were obtained from Toronto Research Chemicals (North York, Canada). [^{3}H]Thiamine (20 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO), and [^{3}H]1-methyl-4-phenylpyridinium (MPP^+) (83.7 Ci/mmol) was obtained from PerkinElmer (Winter Street Waltham, MA). TPP chloride was obtained from MP Biomedicals (Solon, OH). All other reagents used in this study were commercially available and of analytical grade.

In Vitro Transport Study Using cDNA-Transfacted Cells

HEK293 cells that were stably transfected with mouse Oct1, Oct2, Oct3, or empty vector (28) were seeded 72 h before the transport assay in poly-L-lysine- and poly-L-ornithine-coated 12-well plates at a density of 4×10^5 cells per well. The cell culture medium was replaced with culture medium supplemented with 5 mM sodium butyrate at 24 h before the transport assay to induce the expression of the transporters. The transport assay was performed as described previously (29). Briefly, the cells were washed twice and preincubated with Krebs–Henseleit buffer solution (KHBS) at 37°C for 15 min. The KHBS consisted of 118 mM NaCl, 23.8 mM $NaHCO_3$, 4.8 mM KCl, 1.0 mM KH_2PO_4, 1.2 mM $MgSO_4$, 12.5 mM HEPES, 5.0 mM glucose, and 1.5 mM $CaCl_2$ and was adjusted to pH 7.4.

In the presence or absence of TEA (5 mM), uptake was initiated by exchanging preincubated KHBS for [^{3}H]MPP^+ (100 nM) or [^{3}H]thiamine (100 nM) dissolved in the KHBS at 37°C. Uptake was terminated at the designated times by the addition of ice-cold KHBS after the removal of the incubation buffer. The cells were solubilized with NaOH overnight at 4°C, then neutralized with HCl. The radioactivity in the aliquots was measured using liquid scintillation counting. The protein concentration was determined using the Lowry method (30).

Kinetic Analyses

The kinetic parameters were calculated using the following equation:

$$v = V_{max} \times S / (K_m + S) + P_{dif} \times S \quad (1)$$

where P_{dif} is the nonsaturable uptake clearance, v is the uptake velocity of the substrate, V_{max} is the maximum uptake rate, K_m is the Michaelis constant, and S is the substrate concentration in the medium. Fitting was performed using a nonlinear least-squares method using the MULTI program (31).

Isolation of Mouse Hepatocytes from Oct1/2(+/+) and Oct1/2(−/−) Mice

Hepatocytes from the liver of Oct1/2(+/+) and Oct1/2(−/−) mice were prepared by the collagenase perfusion method with minor modification of the protocol for rat hepatocytes preparation (32). The liver was perfused by a solution containing 1 mM EGTA in Earle's balanced salt solution without Ca^{2+}/Mg^{2+}, and then by a solution containing 1 mg/ml collagenase IV with Ca^{2+}/Mg^{2+} in Hanks' balanced salt solution at a flow rate of 6 ml/min. The isolated hepatocyte suspension was obtained after mechanical dissociation, filtration, and low-speed centrifugation. Cell viability was measured (>75%) by trypan blue exclusion test.

Determination of the Uptake of [^{3}H]Thiamine by Mouse Hepatocytes

Isolated hepatocytes were suspended in KHBS and stored on ice. The hepatocytes (10^6 cells) were preincubated for 3 min at 37°C and then the uptake was initiated by adding test compounds, [^{3}H]estradiol-17β-glucuronide, [^{3}H]MPP^+ and [^{3}H]thiamine. After a designated time period, the reaction was terminated by separating the cells from the medium using a centrifugal filtration technique. An 80-μL aliquot of incubation mixture was placed in a 0.25-mL centrifuge tube (Sarstedt, Numbrecht, Germany) containing 50 μL of 2 M NaOH under a 100-μL layer of an oil mixture (density = 1.05, mixture of silicone oil and mineral oil; Sigma-Aldrich) and then centrifuged for 10 s in a microfuge. The radioactivity associated with the cell and medium specimens was measured using a liquid scintillation counter (LS6000SE; Beckman Coulter, Brea, CA).

In Vitro Determination of the Blood-to-Plasma Concentration Ratio

Fresh blood was spiked with four concentrations (0.01, 0.5, 3, and 15 μM) of thiamine-d_3 in triplicates and incubated for 30 min at 37°C. The blood samples were centrifuged to obtain the plasma (2000×*g*, 10 min, 4°C). The concentrations of thiamine-d_3 in the blood (C_b) and in plasma (C_p) were measured using a liquid chromatograph-tandem mass spectrometer (LC-MS/MS). The blood-to-plasma concentration ratio (R_b) was calculated as follows:

$$R_b = C_b/C_p \quad (2)$$

Animal Experiments

The animal studies were performed using methods approved by the Institutional Animal Care and Use Committee of the University of Tokyo and Taisho Pharmaceutical Company for the knockout mouse experiment (Experiments 1 and 2) and the pyrimethamine experiment (Experiment 3), respectively. Oct1 and Oct2 gene-knockout (Oct1/2(−/−)) mice (33,34) were obtained from Taconic Farms (Germantown, NY). We used FVB/Njcl mice (Clea Japan, Tokyo, Japan) as Oct1 and Oct2 gene-wild type controls (Oct1/2(+/+)). The mice were housed in an air-conditioned room set to a 12-h light/dark cycle, and given access *ad libitum* to water and a standard laboratory diet.

Male mice were used at an age of 22 weeks (Experiments 1 and 2) or 7 weeks (Experiment 3). After anesthesia with isoflurane, thiamine-d_3 in saline was infused *via* the jugular vein for 80 min at 1 and 100 nmol/min/kg in Experiments 1 and 2, respectively. Blood samples were collected in a tube containing EDTA as an anticoagulant at 10, 20, 40, and 80 min after administration and the samples were centrifuged to obtain the plasma. At the final sampling point, bladder urine was also collected; after the animals were sacrificed, their kidney, liver, brain, duodenum, jejunum, ileum, and colon were removed and weighed, then homogenized in 4 volumes of ice-cold water. In Experiment 3, a bolus dose of pyrimethamine (5 mg/kg, in 10% hydroxypropyl-β-cyclodextrin) was administered *via* the jugular vein 10 min before the infusion of thiamine-d_3 (100 nmol/min/kg), and the plasma and urine samples were obtained as described above. For the milk secretion study, milk was collected from nursing female Oct1/2(+/+) and Oct1/2(−/−) mice once a day for 3 days as reported previously (35). After milking on the final day, blood was immediately drawn from the jugular vein. Samples were frozen and stored at −80°C until analysis.

Quantification of Thiamine and Thiamine-d_3 Using LC-MS/MS

Blood sample (50 μL) was mixed with 50 μL of 1.2 M ice-cold perchloric acid and kept at 0°C for 15 min. Thereafter this mixture was centrifuged for 4 min at 10,000×*g*. Fifty microliter of supernatant was mixed with 50 μL of 0.6 M KOH/1.8 M potassium acetate for neutralization; then the mixture was centrifuged for 4 min at 10,000×*g* for desalting. A quadruple volume of acetonitrile/methanol (9:1, v/v) containing an internal standard was added to the supernatant from blood sample and to the other biological samples. The mixture was vortexed and centrifuged for 10 min at 3639×*g*, and the supernatant was injected into a LC-MS/MS. For thiamine quantification, the LC system used was a Shimadzu LC-30 AD (Kyoto, Japan) equipped with an XBridge HILIC column (3.5 μm, 4.6×50 mm; Waters, Milford, MA). The mobile phase consisted of 10 mM ammonium acetate (pH 5.0) as solvent A and acetonitrile as solvent B, and it was delivered at a rate of 1.0 mL/min using 90% solvent B between 0 and 0.5 min followed by a linear gradient to 80% B at 2.2 min, 60% B at 2.7 min, 50% B at 4.5 min and then isocratic elution at 50% B for 0.5 min. MS data were acquired using an AB Sciex TripleQuad 5500 tandem mass spectrometer (Foster City, CA) equipped with an electrospray ionization source. The analytes were detected by exploiting the transitions of m/z 265→122 for thiamine, m/z 268→125 for thiamine-d_3, and m/z 283→122 for beclotiamine (an internal standard). For pyrimethamine quantification, the LC system that was used was an Agilent HP1100 system (Agilent Technologies, Santa Clara, CA) equipped with a Shim-pack XR-ODS column (2.2 μm, 3.0×30 mm; Shimadzu). The mobile phase consisted of 0.1% formic acid as solvent A and acetonitrile as solvent B, and it was delivered at a rate of 1.3 mL/min using 2% solvent B at 0 min, followed by a linear gradient to 98% B at 1.0 min and then an isocratic elution at 98% B for 0.3 min. MS data were acquired using an AB Sciex API3000 tandem mass spectrometer equipped with an electrospray ionization source. The analytes were detected by exploiting the transitions of m/z 249→177 for pyrimethamine and m/z 260→116 for propranolol (an internal standard).

Quantification of Total Thiamine

The total thiamine concentration including TPP and TMP was measured by modifying the enzymatic hydrolysis method reported by Wielders *et al.* (36). After thawing tissue homogenate samples, 20 μL were vortexed with 20 μL of 1.2 M ice-cold perchloric acid and kept at 0°C for 15 min. Thereafter this mixture was centrifuged for 10 min at 10,000×*g*. Twenty microliter of supernatant was mixed with 10 μL of internal standard solution (1 μM in saline) and 20 μL of 0.6 M KOH/1.8 M potassium acetate for neutralization; then the mixture was centrifuged for 10 min at 10,000×*g* for desalting. The supernatant (25 μL) was mixed with 25 μL of 4 mg/mL acid phosphatase in saline. Enzymatic hydrolysis was allowed to occur during overnight at room temperature. The final pH was about pH5. An aliquot of acetonitrile/methanol (9:1, v/v, 200 μL) was added, the mixture was vortexed and centrifuged for 10 min at 3639×*g*, and the supernatant was finally subjected to LC-MS/MS under the conditions used for thiamine quantification.

Pharmacokinetic Analysis

The total body clearance with regard to the plasma concentration (CL_{plasma}) was calculated by dividing the infusion rate by the plasma concentration at 80 min after the continuous infusion (C_{80min}). Since the plasma concentration did not reach a plateau during the study in Oct1/2(−/−) mice,

CL_{plasma} was represented as $CL_{plasma,app}$. The renal clearance (CL_R) was calculated by dividing the urinary excretion amount (X_{urine}) from time zero to 80 min by the corresponding area under the plasma concentration–time curve from time zero to 80 min ($AUC_{0–80\ min}$). $AUC_{0–80\ min}$ was calculated using the trapezoidal rule.

Statistical Analysis

Data are presented as the mean ± S.E.M. The statistical analysis was performed using Student's unpaired *t*-test to identify significant differences between the two groups. For the *in vitro* inhibition study, ANOVA, followed by Tukey's multiple comparison test, were performed. A value of $P<0.05$ was considered statistically significant.

RESULTS

Uptake of Thiamine in cDNA Transfectants

The uptakes of [^{3}H]MPP^+ (a prototypical substrate for Oct1-3; 100 nM) in Oct1-, Oct2- or Oct3-expressing HEK293 cells were significantly greater than that in empty vector-transfected control cells and were inhibited by TEA (Fig. 1a). The uptakes of [^{3}H]thiamine (100 nM) in Oct1- or Oct2-expressing cells were also significantly greater than that in empty vector-transfected control cells and were inhibited by TEA, whereas the uptake in Oct3-expresing cells was similar to that in the empty vector-transfected cells (Fig. 1a). The uptake of thiamine by Oct1- or Oct2-expressing cells consisted of a saturable uptake and a non-saturable uptake (Fig. 1b and c). The kinetic parameters obtained by fitting were as follows: K_m 36.3±9.2 and 22.6±4.4 μM; V_{max} 353±52 and 258±36 pmol/min/mg protein; and P_{dif} 0.454±0.048 and 2.09±0.11 μL/min/mg protein in the Oct1- and Oct2-expressing cells, respectively.

Uptake of Thiamine in Mouse Hepatocytes

The uptake of representative substrates for multispecific organic anion transporters (Oatps) and Oct1, estradiol-17β-glucuronide and MPP^+, respectively, was determined (Supplemental Material Fig. S1). The uptake of estradiol-17β-glucuronide was similar between Oct1/2(+/+) and Oct1/2(−/−) mice, and the uptake was significantly inhibited by the representative inhibitor, rifampicin. On the other hand, the uptake of MPP^+ was significantly decreased in the hepatocytes from Oct1/2(−/−) mice compared with that from Oct1/2(+/+) mice; however, the uptake of MPP^+ remained sensitive to TEA.

The uptake of [^{3}H]thiamine was determined in the hepatocytes. The concentration of [^{3}H]thiamine (100 nM) was selected on the basis of a previous report by Yoshioka, where two processes were detected with K_m of 1.3 and 34 μM in rat hepatocytes (24). The excess concentration (500 μM) was high enough to saturate Oct1-mediated uptake based on the K_m value determined in this study. Hepatocytes from the liver of Oct1/2(−/−) mice showed a significant reduction in the uptake of [^{3}H]thiamine compared with those from Oct1/2(+/+) mice at tracer concentration (Fig. 2a). [^{3}H]Thiamine uptake by the hepatocytes from Oct1/2(+/+) mice was decreased in the presence of excess non-radiolabeled thiamine (500 μM) or TEA (Fig. 2b). [^{3}H]Thiamine uptake by the hepatocytes from Oct1/2(−/−) mice was also decreased in the presence of excess non-radiolabeled thiamine (500 μM) or TEA, but the decrease was not statistically significant.

Effect of Oct1/2 Deficiency on the Endogenous Thiamine in the Plasma

Mice were fed mouse food (CE-2, CLEA Japan, Inc. Tokyo, Japan) which contains thiamine as vitamin. Food-derived thiamine was referred to as endogenous thiamine in this study. The endogenous thiamine concentrations in plasma were 0.0972±0.0139 and 0.559±0.060 nmol/mL ($n=5$) in male Oct1/2(+/+) and Oct1/2(−/−) mice, respectively ($P<0.05$).

Effect of Oct1/2 Deficiency on Thiamine-d_3 Clearance Under Physiological Conditions (Experiment 1)

The plasma concentration-time profiles of thiamine-d_3 in male Oct1/2(+/+) and Oct1/2(−/−) mice after the continuous infusion of thiamine-d_3 at a low dose (1 nmol/min/kg) are shown in Fig. 3. X_{urine} and the pharmacokinetic parameters are listed in Table I (Experiment 1). The plasma concentrations of thiamine-d_3 in Oct1/2(−/−) mice were significantly higher than those in Oct1/2(+/+) mice. Given that the plasma concentrations of thiamine-d_3 did not reach a plateau during the study in Oct1/2(−/−) mice, the $CL_{plasma,app}$ apparently overestimated the true CL_{plasma}. The CL_{plasma} was decreased by more than 80% in Oct1/2(−/−) mice compared with those in Oct1/2(+/+) mice.

The CL_R accounted for a negligible portion of the CL_{plasma} (0.36% in Oct1/2(+/+) mice). There was no significant difference in X_{urine} between Oct1/2(+/+) and Oct1/2(−/−) mice. The mean value of the CL_R was smaller in Oct1/2(−/−) mice than in Oct1/2(+/+) mice, however, without statistical significance (Table I (Experiment 1)).

Blood-to-Plasma Concentration Ratio of Thiamine-d_3

The R_b of thiamine-d_3 was 2.68 at 0.085 μM of the effective concentration (endogenous thiamine, 0.075 μM, plus

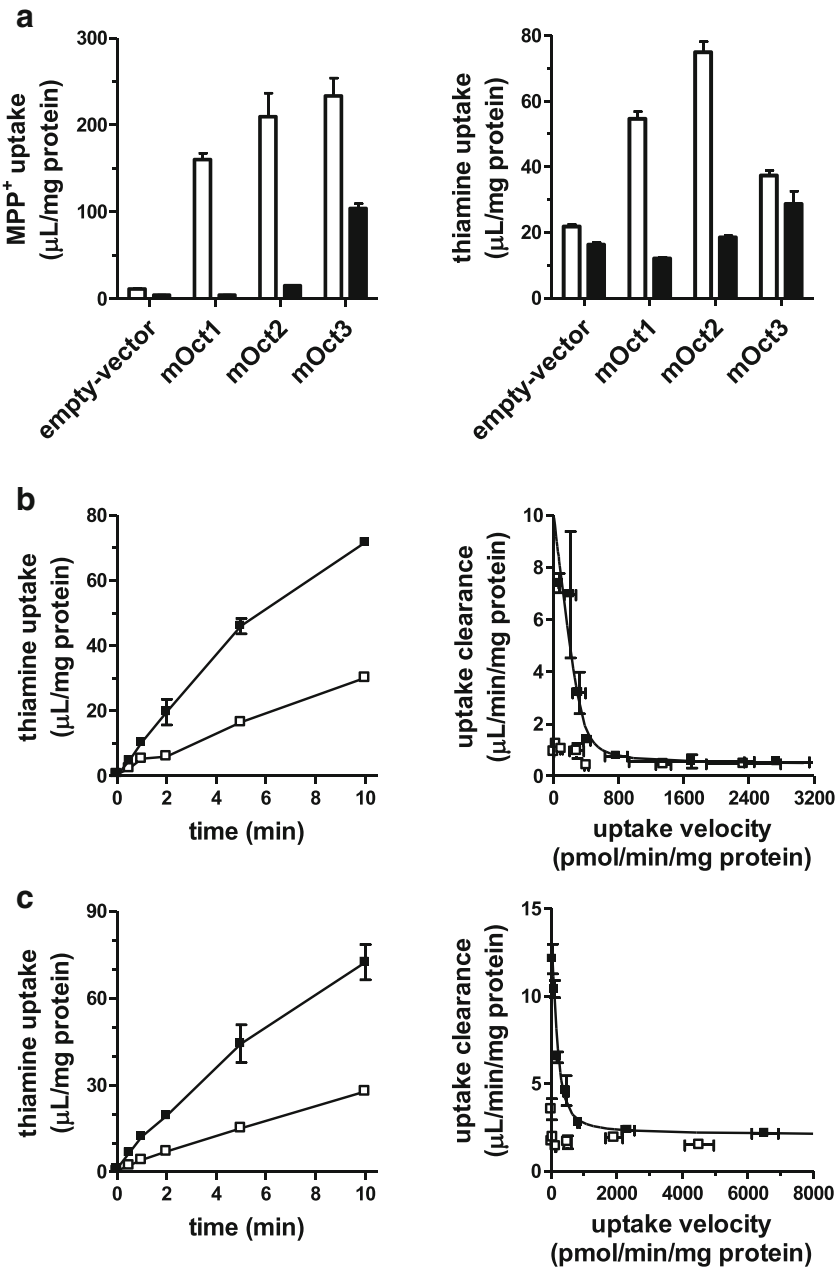

Fig. 1 Uptake of [^{3}H]thiamine by HEK293 cells expressing Oct1, Oct2, or Oct3. (**a**) HEK293 cells expressing Oct1, Oct2, or Oct3 or that were transfected with an empty vector were incubated with [^{3}H]MPP$^+$ (100 nM) or [^{3}H]thiamine (100 nM) at 37°C for 10 min in the presence (*closed bars*) or absence (*open bars*) of TEA (5 mM). The uptakes of [^{3}H]thiamine (100 nM) by empty-vector cells (*open squares*), (**b**) Oct1-HEK cells, and (**c**) Oct2-HEK cells (*closed squares*) were determined at the designated time points at 37°C. The concentration dependence of the uptake of thiamine by empty-vector cells (*open squares*), (**b**) Oct1-HEK cells, and (**c**) Oct2-HEK cells (*closed squares*) is shown as Eadie-Hofstee plots. The thiamine concentrations were 3–3000 μM. Data are expressed as the mean ± S.E.M. ($n = 3$).

thiamine-d_3, 0.01 μM) in Oct1/2(+/+) mice (Fig. 4). The R_b in Oct1/2(−/−) mice was 0.78 at 0.48 μM (endogenous thiamine, 0.47 μM, plus thiamine-d_3, 0.01 μM). The R_b values in both Oct1/2(+/+) and Oct1/2(−/−) mice were approximately 1 at concentrations greater than 3 μM, and no difference between R_b of Oct1/2(+/+) and Oct1/2(−/−) mice was observed.

Effect of Oct1/2 Deficiency on Thiamine-d_3 Clearance Under Excess Intake Conditions (Experiment 2)

The plasma concentration–time profiles of thiamine-d_3 in male Oct1/2(+/+) and Oct1/2(−/−) mice after the continuous infusion of thiamine-d_3 at a high dose (100 nmol/min/kg) are shown in Fig. 5a. X_{urine} and the pharmacokinetic parameters are listed in Table I (Experiment 2). Under excess intake conditions, the urinary excretion accounted for 67% of the systemic elimination of thiamine-d_3 in Oct1/2(+/+) mice. The plasma concentrations in Oct1/2(−/−) mice were significantly higher than those in Oct1/2(+/+) mice. Since it did not reach a plateau during the study, causing overestimation of the true CL_{plasma} by $CL_{plasma,app}$ in Oct1/2(−/−) mice, the CL_{plasma} decreased by more than 78% in Oct1/2(−/−) mice compared with those in Oct1/2(+/+) mice. There was no statistical difference in the X_{urine} between the Oct1/2(+/+) and Oct1/2(−/−) mice. CL_R decreased by 79% in Oct1/2(−/−) mice.

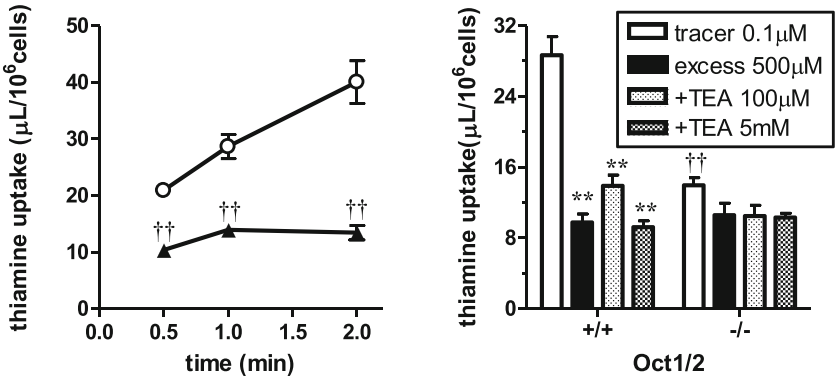

Fig. 2 Uptake of [^{3}H]thiamine by freshly isolated hepatocytes from Oct1/2(+/+) and Oct1/2(−/−) mice. (**a**) Hepatocytes were freshly isolated from Oct1/2(+/+) and Oct1/2(−/−) mice (*open and closed symbols*, respectively). Uptake of [^{3}H]thiamine (100 nM) by the isolated mouse hepatocytes was determined at 37°C at the designated time-points. (**b**) Uptake of [^{3}H]thiamine by isolated mouse hepatocytes for 1 min was determined at 37°C at thiamine concentrations of 100 nM and 500 μM. The effect of TEA on the uptake of [^{3}H]thiamine (100 nM) at the designated concentrations was examined. Data are expressed as the mean ± S.E.M. ($n=3$). ** $P<0.01$ *versus* control condition, †† *versus* Oct1/2(+/+) mouse

Effect of Mate1 Inhibition by Pyrimethamine on Thiamine-d_3 Clearance Under Excess Intake Conditions (Experiment 3)

Pyrimethamine, a selective MATE inhibitor, was administered (5 mg/kg) to normal male mice in the pyrimethamine-treatment group at 10 min before beginning the infusion with thiamine-d_3 (100 nmol/min/kg). At 80 min after beginning the infusion, the total concentration of pyrimethamine in the kidney and plasma was 51.1 ± 3.4 and 5.55 ± 0.81 μM, respectively. The concentrations of unbound pyrimethamine in the kidney and plasma, calculated as the product of total concentrations and previously reported unbound fractions [0.026 and 0.081 in the kidney and plasma (28)] were 1330 ± 87 and 449 ± 66 nM, respectively. The concentration of unbound pyrimethamine in the kidney was much higher than the K_i value for mouse Mate1 [145 nM (28)], and the concentration of unbound pyrimethamine in the plasma was much lower than the K_i values for mouse Oct1 and Oct2 [3.6 and 6.0 μM, respectively (28)]. Thus, pyrimethamine appeared to inhibit Mate1 but not Oct1 or Oct2.

The plasma concentration–time profiles of thiamine-d_3 in the control and pyrimethamine-treated mice are shown in Fig. 5b. X_{urine} and the pharmacokinetic parameters are listed in Table I (Experiment 3). The plasma concentrations in pyrimethamine-treated mice were significantly higher compared with those in control mice. The CL_{plasma} and CL_R were decreased by 47% and 58%, respectively, in the pyrimethamine-treated mice compared with those in the controls.

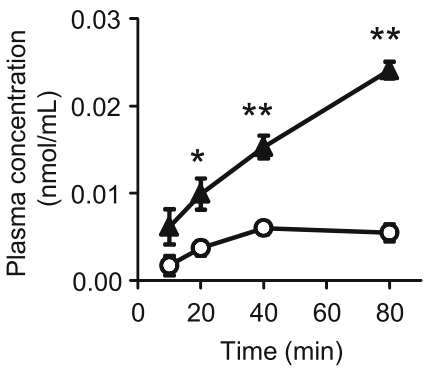

Fig. 3 Effect of Oct1/2 deficiency on the plasma concentrations of thiamine-d_3 in male mice after administration at a low dose. The plasma concentrations of thiamine-d_3 were determined in Oct1/2(+/+) mice (*open symbols*) and Oct1/2(−/−) mice (*closed symbols*) using LC-MS/MS. Thiamine-d_3 was administered to mice *via* an intravenous infusion at a dosing rate of 1 nmol/min/kg. Blood specimens were collected *via* the jugular vein at the designated times, and bladder urine was collected at 80 min after the start of infusion. Each point represents the mean value and S.E.M. ($n=4$). * $P<0.05$; ** $P<0.01$ *versus* Oct1/2(+/+).

Effect of Oct1/2 Deficiency on the Tissue and Plasma Concentrations of Total Thiamine

Authentic standards of TMP and TPP (1 and 10 μM) were quantitatively hydrolyzed by acid phosphatase overnight under ambient conditions (data not shown). The thiamine concentration after the enzymatic hydrolysis was defined as the total thiamine concentration. The concentrations of endogenous total thiamine and exogenous total thiamine-d_3 in plasma, kidney, liver, duodenum, jejunum, ileum, colon, and brain collected at 80 min after the start of thiamine-d_3 infusion in male Oct1/2(+/+) and Oct1/2(−/−) mice are listed in Table II. No differences in the total thiamine and thiamine-d_3 levels were observed in the kidney, liver, and brain, but the gastrointestinal tract levels of total thiamine and thiamine-d_3 were significantly lower in Oct1/2(−/−) mice than in Oct1/2(+/+) mice (0.6- to 0.8-fold and 0.3- to 0.4-fold, respectively). The total thiamine and thiamine-d_3 concentrations in the plasma were significantly higher in Oct1/2(−/−) mice than in Oct1/2(+/+) mice (2.7-fold and 3.2-fold higher, respectively) (Table II). Plasma contains TMP as a phosphorylated ester of thiamine, but the amount of TPP is minimal

Table I Comparison of Pharmacokinetic Parameters of Thiamine-d_3 Between Male Oct1/2(+/+) and Oct1/2(−/−) Mice, and Between Control and Pyrimethamine-Treated Male Mice

Parameter	Unit	Experiment 1			Experiment 2			Experiment 3		
		Oct1/2(+/+)	Oct1/2(−/−)		Oct1/2(+/+)	Oct1/2(−/−)		Control	PYR (5 mg/kg)	
Infusion rate	nmol/min/kg	1	1		100	100		100	100	
C_{80min}	nmol/mL	0.00546 ± 0.00098	0.0241 ± 0.0010	**	3.32 ± 0.88	14.4 ± 2.6	**	1.87 ± 0.25	3.38 ± 0.13	**
$AUC_{0-80min}$	nmol·min/mL	0.362 ± 0.047	1.15 ± 0.06	**	177 ± 33	654 ± 156	*	121 ± 13	210 ± 12	**
X_{urine}	nmol	0.00991 ± 0.00471	0.00957 ± 0.00289	n.s.	169 ± 55	105 ± 25	n.s.	123 ± 11	90.6 ± 11.9	n.s.
CL_{plasma}	mL/min/kg	209 ± 50	N/A		38.3 ± 11.4	N/A		56.3 ± 7.1	29.7 ± 1.1	*
$CL_{plasma,app}$[a]	mL/min/kg	N/A	41.6 ± 1.6		N/A	8.49 ± 2.20		N/A	N/A	
CL_R	mL/min/kg	0.710 ± 0.256	0.240 ± 0.064	n.s.	24.8 ± 6.9	5.13 ± 1.04	*	34.9 ± 4.4	14.7 ± 2.3	**

Each value was determined from the data shown in Figs. 3 and 5. Each value represents the mean ± S.E.M. ($n = 4$ or 5)

$AUC_{0-80min}$ the area under the plasma concentration–time curve from time zero to 80 min, *C_{80min}* plasma concentration at 80 min after the continuous infusion, *CL_{plasma} and $CL_{plasma,app}$* total body clearance with regard to the plasma concentration, *CL_R* renal clearance, *N/A* not applicable, *PYR* pyrimethamine

* $P < 0.05$; ** $P < 0.01$; n.s., not statistically significant *versus* Oct1/2(+/+) or control

[a] Estimated under unsteady-state conditions

(37). The increases of thiamine in the plasma after hydrolysis, which was defined as TMP, were calculated to be 0.119 ± 0.027 and 0.0369 ± 0.0115 nmol/mL in Oct1/2(+/+) and Oct1/2(−/−) mice, respectively; this difference was statistically significant ($P < 0.05$, $n = 5$).

Effect of Oct1/2 Deficiency on the Plasma and Breast-Milk Concentrations of Thiamine in Lactating Mice

The plasma and breast-milk concentrations of endogenous thiamine in lactating female Oct1/2(+/+) and Oct1/2(−/−) mice are shown in Fig. 6 and Table S1 (supplementary material). The plasma concentration of thiamine in Oct1/2(−/−) mice was significantly 2.8-fold higher compared with those in Oct1/2(+/+) mice. Nevertheless, the breast-milk thiamine level in Oct1/2(−/−) mice was 9.8-fold lower than that in Oct1/2(+/+) mice. Consequently, the milk-to-plasma concentration ratio (M/P) was 28-fold lower in Oct1/2(−/−) mice.

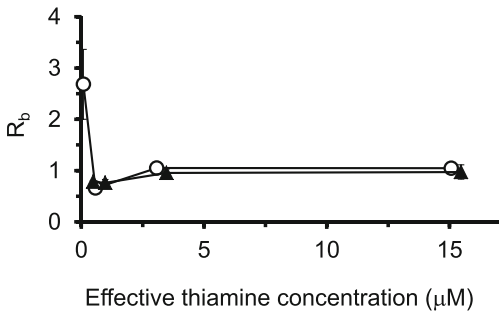

Fig. 4 The blood-to-plasma concentration ratio of thiamine-d_3 in male Oct1/2(+/+) and Oct1/2(−/−) mice. Fresh blood samples obtained from Oct1/2(+/+) (open circles) and Oct1/2(−/−) mice (closed triangles) were incubated with thiamine-d_3 (0.01, 0.5, 3 and 15 μM) at 37°C for 30 min. The horizontal axis represents the effective thiamine concentrations which represent the sum of the concentrations of thiamine-d_3 and the endogenous thiamine. The endogenous thiamine concentration were 0.075 and 0.47 μM in Oct1/2(+/+) and Oct1/2(−/−) mice, respectively. The vertical axis represents blood-to-plasma concentration ratio (R_b) of thiamine-d_3. Data are expressed as the mean ± S.E.M. ($n = 3$).

DISCUSSION

The purpose of the present study was to investigate the role of Octs in the plasma clearance, renal clearance, and tissue distribution of thiamine as well as in secretion of thiamine in milk and the role of Mate1 in the renal clearance of thiamine in mice.

We obtained *in vitro* evidence supporting the hypothesis that thiamine is a definite substrate for the tissue-specific isoforms, Oct1 and Oct2, but not for the ubiquitous isoform, Oct3 (Fig. 1). The K_m values of thiamine for Oct1 and Oct2 (36.3 and 22.6 μM, respectively), were greater than those for the thiamine-specific transporters, THTR-1 and THTR-2 [2.5 μM (14) and 27 nM (15), respectively], indicating that Oct1 and Oct2 act as low-affinity thiamine transporters.

To elucidate the importance of Oct1 and Oct2 in thiamine disposition, *in vitro* and *in vivo* studies using Oct1/2(−/−) mice were conducted. The hepatocytes isolated from the liver of Oct1/2(−/−) mice showed a marked reduction in the saturable component of thiamine uptake (Fig. 2). Oct1/2(−/−) mice showed a 5.8-fold higher plasma concentration of endogenous thiamine than that of Oct1/2(+/+) mice, whereas the plasma level of TMP was significantly lower in Oct1/2(−/−) mice. To obtain kinetic data, an *in vivo* study using thiamine-d_3 was

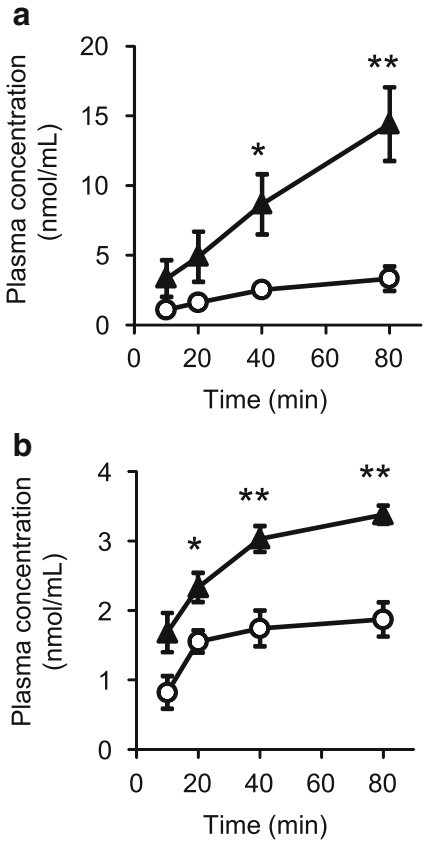

Fig. 5 Effect of Oct1/2 deficiency and Mate 1 inhibition on the plasma concentrations of thiamine-d_3 in male mice after administration at a high dose. (**a**) The plasma concentrations of thiamine-d_3 were determined in Oct1/2(+/+) (*open symbols*) and Oct1/2(−/−) mice (*closed symbols*). Thiamine-d_3 was administered to mice *via* an intravenous infusion at a dosing rate of 100 nmol/min/kg. Blood specimens were collected *via* the jugular vein at the designated times, and bladder urine was collected at 80 min after the start of infusion. Each point represents the mean value and S.E.M. ($n = 4$ or 5). * $P < 0.05$; ** $P < 0.01$ *versus* Oct1/2(+/+). (**b**) The plasma concentrations of thiamine-d_3 were determined in control (*open symbols*) and pyrimethamine-pretreatment mice (*closed symbols*). Pyrimethamine (5 mg/kg) was administered to mice *via* a bolus injection 10 min before the start of the intravenous infusion of thiamine-d_3 (100 nmol/min/kg). Blood specimens were collected *via* the jugular vein at the designated times, and bladder urine was collected at 80 min after the start of infusion. Each point represents the mean value and S.E.M. ($n = 4$). * $P < 0.05$; ** $P < 0.01$ *versus* control.

conducted in Oct1/2(+/+) and Oct1/2(−/−) mice. After continuous infusion of thiamine-d_3 at 1 nmol/min/kg, the plasma levels of thiamine-d_3 were more than 20-fold lower than the basal level of endogenous thiamine (Fig. 3), suggesting that the disposition of thiamine-d_3 was under normal conditions. Urinary excretion negligibly contributed to the systemic elimination of thiamine-d_3 (Table I-Experiment 1), suggesting that Oct1 defect greatly contributes to this change compared with Oct2 defect. The plasma concentration of thiamine-d_3 was significantly higher in Oct1/2(−/−) mice (Fig. 3). Given that $CL_{plasma,app}$ overestimated the CL_{plasma} in Oct1/2(−/−) mice, the CL_{plasma} of thiamine-d_3 was decreased by more than 80% in Oct1/2(−/−) mice compared with that in Oct1/2(+/+) mice (Table I-Experiment 1). It is debatable whether the magnitude of the reduction in CL_{plasma} represents the fraction of Oct1-mediated transport. First, the fraction of the clearance in the liver and intestine where Oct1 is expressed in the non-renal clearance was not determined in this study. This data is needed to compare the intrinsic clearance in the responsible tissues between Oct1/2(+/+) and Oct1/2(−/−) mice. Second, in addition to the reduction in Oct-mediated tissue uptake, elevation of basal thiamine plasma concentration in Oct1/2(−/−) mice may affect the clearance and tissue distribution of thiamine-d_3 because of non-linearity.

Because the erythrocytes show saturable uptake of thiamine mediated by THTR-1, it is possible that the R_b of thiamine-d_3 in Oct1/2(−/−) mice is not identical to that in Oct1/2(+/+) mice. In fact, the R_b of thiamine-d_3 determined at physiologically relevant concentrations in Oct1/2(+/+) and Oct1/2(−/−) mice (~0.1 and 0.5 μM, respectively) was 3-fold lower in Oct1/2(−/−) mice than in Oct1/2(+/+) mice (Fig. 4). This is presumably attributable to the saturation of thiamine uptake along with an increase in the basal thiamine concentration in Oct1/2(−/−) mice because the R_b determined in Oct1/2(+/+) mice decreased at a concentration equivalent to the basal plasma level in Oct1/2(−/−) to a level similar to that in Oct1/2(−/−) mice (Fig. 4). Non-linearity in R_b will make the difference between the blood concentrations of thiamine in Oct1/2(+/+) and Oct1/2(−/−) mice smaller than that between the plasma concentrations. Hence, the non-linearity in uptake by erythrocytes results in limitation of the amount of thiamine in the body.

Considering the bidirectional transport properties of Octs and extensive reabsorption of thiamine under normal conditions, Oct1/2 is speculated to mediate the basolateral efflux of thiamine in the kidney. However, Oct1/2(−/−) mice had almost reduced CL_R, although the difference did not reach statistical significance (Table I-Experiment 1). The transporter responsible for thiamine reabsorption remains to be determined. Immunohistochemical staining of THTR-1 and THTR-2 in the human kidney demonstrated that the proteins are exclusively expressed in the brush border membrane of the renal tubules of the cortex (17). In MDCK cells, THTR-2 was exclusively expressed in the apical membrane, whereas THTR-1 was expressed on both the basal and apical membranes (38). Thus, Larkin *et al.* added THTR-1 to the basolateral membrane (17), and speculated that THTR-1 is a candidate for mediating the efflux of thiamine to the blood circulation in conjunction with apical THTR-1 and THTR-2.

To clearly demonstrate the tubular secretion of thiamine *via* Oct1, Oct2 and Mate1, the CL_R of thiamine was determined in the presence of excess thiamine to suppress the reabsorption (Table I-Experiment 2). When excess thiamine-d_3 (100 nmol/min/kg) was administered to mice, urinary excretion significantly contributed to the elimination from

Table II Tissue Concentrations of Total Endogenous Thiamine and Total Exogenous Thiamine-d_3 in Male Oct1/2(+/+) and Oct1/2(−/−) Mice

Tissue	Total endogenous thiamine (nmol/mL or nmol/g tissue)				Total exogenous thiamine-d_3 (pmol/mL or pmol/g tissue)			
	Oct1/2(+/+)	Oct1/2(−/−)	Fold change		Oct1/2(+/+)	Oct1/2(−/−)	Fold change	
Plasma	0.216 ± 0.028	0.588 ± 0.061	2.7	**	8.54 ± 1.11	27.3 ± 0. 4	3.2	**
Kidney	38.2 ± 2.3	37.0 ± 0.9	1.0	n.s.	265 ± 27	249 ± 33	0.9	n.s.
Liver	28.1 ± 2.0	25.9 ± 1.7	0.9	n.s.	148 ± 16	196 ± 39	1.3	n.s.
Duodenum	10.1 ± 0.7	6.16 ± 0.40	0.6	**	88.2 ± 25.1	25.0 ± 4.5	0.3	*
Jejunum	12.3 ± 1.5	7.33 ± 0.71	0.6	*	88.1 ± 23.7	24.6 ± 3.8	0.3	*
Ileum	8.62 ± 0.83	5.64 ± 0.81	0.7	*	44.7 ± 10.6	16.0 ± 3.9	0.4	*
Colon	7.46 ± 0.35	5.75 ± 0.52	0.8	*	59.7 ± 10.1	16.5 ± 2.4	0.3	**
Brain	7.49 ± 0.67	8.25 ± 0.34	1.1	n.s.	9.44 ± 0.76	9.58 ± 0.96	1.0	n.s.

Thiamine-d_3 was intravenously administered to male Oct1/2(+/+) mice and Oct1/2(−/−) mice *via* a constant infusion at a dosing rate of 1 nmol/min/kg in Experiment 1; each plasma and tissue sample was collected 80 min after the continuous infusion

Each value represents the mean ± S.E.M. ($n = 4$). * $P < 0.05$ ** $P < 0.01$; *n.s.* not statistically significant when compared with Oct1/2(+/+)

the systemic circulation (>67%) and the CL_R became greater than the glomerular filtration rate [GFR; previously reported values, 17 mL/min/kg (28) and 14 mL/min/kg (39)]. Oct1/2(−/−) mice and mice treated with pyrimethamine had a significantly reduced CL_R of thiamine-d_3 (Table I-Experiments 2 and 3), supporting that Oct1/2 and Mate1 played a role in the tubular secretion of thiamine. Because the CL_R in pyrimethamine-treated mice was greater than that in Oct1/2(−/−) mice, pyrimethamine may not have diminished the tubular secretion at the dose tested. There are two possible reasons for this: insufficient concentration of pyrimethamine for complete inhibition of Mate1 and/or involvement of unknown transporters insensitive to pyrimethamine with lesser contribution than that by Mate1.

The present study demonstrated the importance of Oct1 in determining the plasma concentration of thiamine under normal conditions and that of Oct1, Oct2 and Mate1 in the tubular secretion of thiamine in the presence of excess thiamine. Therefore, thiamine is expected to be a probe substrate for evaluating the inhibitory potential of drug candidates on OCT1, and renal tubular secretion *via* OCT2 and MATE1/2-K, although further validation studies are needed.

To address whether defect in Oct1/2 affect the vitamin B_1 nutritional status in tissues, the tissue concentrations of total thiamine, including thiamine and its phosphorylated esters, were measured (Table II). The sum of TPP and TMP accounts for more than 90% of the total thiamine in the liver, kidney, and brain (40). The tissue total thiamine concentrations obtained in this study were comparable with those reported previously (40). Unlike the plasma concentration, only small differences in the total thiamine concentrations in the kidney, liver, and brain were observed between Oct1/2(+/+)

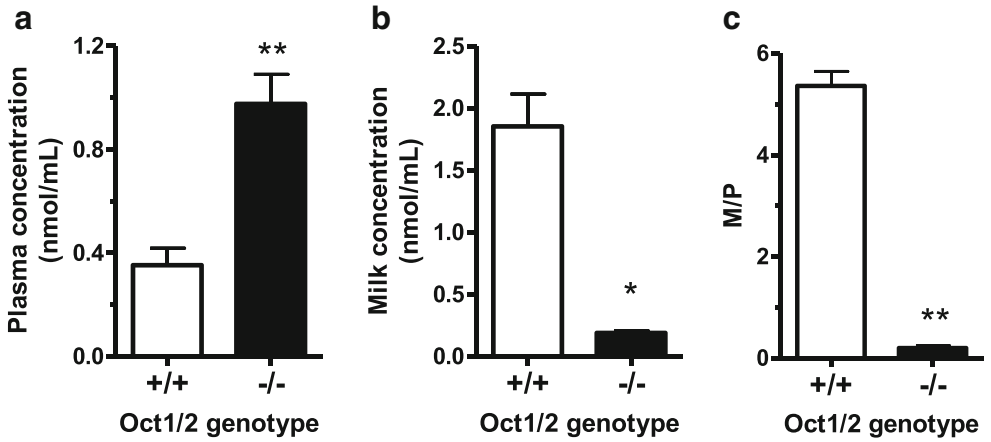

Fig. 6 Effect of Oct1/2 deficiency on the plasma and breast-milk thiamine concentrations in lactating female mice. The plasma (**a**) and breast-milk (**b**) concentrations of thiamine endogenously present in the specimens were determined in female Oct1/2(+/+) and Oct1/2(−/−) mice. M/P (C) represents the milk-to-plasma concentration ratio. Milk was collected from nursing female mice once a day for 3 days, and the mean value was obtained in the individual mouse. After milking on the final day, blood was immediately drawn from the jugular vein. Each point represents the mean value and S.E.M. ($n = 3$). * $P < 0.05$; ** $P < 0.01$ *versus* Oct1/2(+/+).

and Oct1/2(−/−) mice, whereas the concentrations in the small and large intestine were significantly lower in the Oct1/2(−/−) mice. However, the intestinal total thiamine levels in Oct1/2(−/−) mice remained much higher than the tissue levels at which the symptoms of thiamine deficiency become observable (41). Thus, Oct1/2 defects are likely to have a minor impact on the vitamin B_1 nutritional status, at least under normal conditions. The liver and kidney thiamine concentrations probably do no depend on the activities of Octs, because reduction in tissue uptake activity can be compensated for by an increase in the plasma concentration. This observation sharply contrasts with observations of the drug disposition of metformin; its distribution is diminished in the hepatocytes of Oct1(−/−) mice (42). Hepatocytes might maintain the uptake of thiamine from plasma presumably *via* the thiamine transporters Thtr-1 and/or Thtr-2. The uptake of [^{3}H]thiamine in Oct1/2(−/−) mice was decreased in the presence of excess thiamine, although the difference did not reach the statistical significance (Fig. 2). The plasma level of endogenous thiamine was higher than the K_m value of THTR-2 (27 nM (15)), indicating that THTR-2-mediated transport from the blood side become saturated even under physiological conditions; thus, THTR-1 (K_m, 2.5 μM (14)) might have a larger contribution than THTR-2. Reduced folate carrier (RFC, *SLC19A1*) has been proposed as an alternative transport route available for thiamine monophosphate (TMP) from the plasma into some tissues when THTR-1 is mutated (43). Plasma TMP might contribute to the maintenance of thiamine levels in the liver and kidney in Oct1/2(−/−) mice. On the other hand, the gastrointestinal tract might not have a transport protein for the uptake of thiamine from the blood side other than Oct1, since Thtr-1 and/or Thtr-2 transports thiamine from the lumen into blood (44). This rationale might account for the impact of impaired Oct1 and Oct2 on the tissue concentrations of total thiamine. Contradictory to our speculation (41), Han *et al.* demonstrated that OCT1 and Oct1 were localized in the apical membrane in human and mouse intestine, respectively (45). Reduction in the amount of thiamine associated with intestine may be caused by reduced uptake from the lumen of thiamine that was excreted into the bile. Further investigation is required to elucidate the involvement of Oct1 in the intestinal absorption of thiamine. In the brain, which expresses Oct1/2 minimally, no increase in the total thiamine levels was observed despite the 5.8-fold higher plasma thiamine levels in the Oct1/2(−/−) mice, compared with the levels in the Oct1/2(+/+) mice. We assumed that the brain level of thiamine was probably maintained by the saturation of an uptake transporter of thiamine. Indeed, using an *in situ* rat brain perfusion technique, the saturable transport of thiamine into the brain was observed with a K_m of 96 nM (46), indicating total saturation at physiologically relevant plasma concentration of thiamine.

We also found that the M/P value of thiamine was 28-fold lower in lactating Oct1/2(−/−) mice than in Oct1/2(+/+) mice (Fig. 6). The plasma concentration of thiamine in lactating female Oct1/2(−/−) mice was higher than that in the corresponding Oct1/2(+/+) mice. Notably, the milk concentration of thiamine was markedly lower in lactating Oct1/2(−/−) mice compared with that in Oct1/2(+/+) mice. Oct1 is likely responsible for the milk secretion of thiamine, considering that the expression level of Oct1 was much greater than that of Oct2 in the mammary glands of lactating mice (27). Unlike the kidney and liver, the expression of Mate1 was extremely low in the mammary gland (27). The transporter responsible for thiamine efflux in the mammary gland remains unknown. Previously, an ATP binding cassette transporter, BCRP, was shown to mediate the milk secretion of riboflavin in the mammary gland (47). However, the levels of other vitamin B compounds, including thiamine, were not altered in Bcrp(−/−) mice compared with Bcrp(+/+) mice. Other unknown transporters likely mediate the efflux of thiamine into milk. When the total thiamine (thiamine plus TMP) concentrations in the breast milk of 636 lactating women were measured in a Maela refugee camp, the average value was 755 nM (interquartile range, 730–781 nM), but 4.1% of the population had a level of less than 300 nM (48). These observations might be partly related to the presence of functionally deleterious mutations in the *SLC22A1* gene, such as P283L and P341L (49,50).

CONCLUSION

Oct1 is presumably responsible for the uptake of thiamine from the systemic blood in tissues, such as liver, kidney, and intestine, and its decline can accordingly decrease the CL_{plasma} of thiamine. Rapid renal thiamine elimination, where there is excess intake, can be attributed to tubular secretion *via* Oct1/2 and Mate1. Oct1/2 deficiency had a minor impact on the nutritional status of tissues for thiamine, but a major impact on the secretion of thiamine in milk in lactating mice was observed.

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