Impaired Intestinal Vitamin B_1 (Thiamin) Uptake in Thiamin Transporter-2–Deficient Mice

JACK C. REIDLING,[*,§] NILS LAMBRECHT,[‡] MOHAMMAD KASSIR,[*,§] and HAMID M. SAID[*,‡,§,‖]

[*]Departments of Medical Research and [‡]Pathology/Laboratory Medicine, VA Medical Center, Long Beach, California; and [§]Departments of Medicine and [‖]Physiology/Biophysics, University of California College of Medicine, Irvine, California

BACKGROUND & AIMS: Intestinal thiamin uptake process is vital for maintaining normal body homeostasis of the vitamin; in vitro studies suggest that both thiamin transporter-1 (THTR-1) and -2 (THTR-2) are involved. Mutations in THTR-1 cause thiamin-responsive megaloblastic anemia, a tissue-specific disease associated with diabetes mellitus, megaloblastic anemia, and sensorineural deafness. However, in patients with thiamin-responsive megaloblastic anemia, plasma thiamin levels are within normal range, indicating that THTR-2 (or another carrier) could provide sufficient intestinal thiamin absorption. We tested this possibility and examined the role of THTR-2 in uptake of thiamin in the intestine of mice. **METHODS:** THTR-2–deficient mice were generated by *SLC19A3* gene knockout and used to examine intestinal uptake of thiamin in vitro (isolated cells) and in vivo (intact intestinal loops). We also examined intestinal thiamin uptake in THTR-1–deficient mice. **RESULTS:** Intestine of THTR-2–deficient mice had reduced uptake of thiamin compared with those of wild-type littermate mice ($P < .01$); this reduction was associated with a decrease ($P < .01$) in blood thiamin levels in THTR-2–deficient mice. However, intestinal uptake of thiamin in THTR-1–deficient mice was not significantly different from that of wild-type littermate animals. Level of expression of THTR-1 was not altered in the intestine of THTR-2–deficient mice, but level of expression of THTR-2 was up-regulated in the intestine of THTR-1–deficient mice. **CONCLUSIONS: THTR-2 is required for normal uptake of thiamin in the intestine and can fulfill normal levels of uptake in conditions associated with THTR-1 dysfunction.**

Keywords: Thiamin; THTR-1; THTR-2.

Vitamin B_1 (thiamin) is essential for the survival of human beings and other mammals. Thiamin plays a fundamental role in metabolism and energy production as the coenzyme thiamin pyrophosphate, a metabolite required for normal cellular functions, growth, and development.[1] Further, thiamin plays a role in reducing cellular oxidative stress via its role in bridging the glycolytic and the pentose phosphate metabolic pathways.[2,3] Insufficient amounts of dietary thiamin can lead to a variety of clinical abnormalities that include neurologic and cardiovascular disorders.[1,4,5] An inability to synthesize thiamin has generated, in mammals, a reliance on the intestinal epithelium to maintain normal thiamin body homeostasis. By using a variety of human and animal intestinal preparations, the process of intestinal thiamin uptake was shown to occur via a specialized carrier-mediated mechanism (see review by Said[6]). Other studies have shown that both thiamin transporters-1 and -2 (THTR-1 and THTR-2, respectively) are expressed in human and mouse intestine at the protein and messenger RNA (mRNA) levels.[6–8] Further, recent in vitro investigations using a gene-silencing approach (ie, gene-specific small interfering RNA) in cultured human-derived intestinal epithelial Caco-2 cells have shown that both THTR-1 and THTR-2 are involved in thiamin uptake across the apical membrane domain and that together they account for total carrier-mediated uptake of the vitamin.[7] However, confirmation of these findings in a more physiologically relevant setting currently is lacking.

The autosomal-recessive disorder thiamin-responsive megaloblastic anemia (TRMA) is caused by mutations in THTR-1.[9–11] TRMA is associated with megaloblastic anemia, diabetes mellitus, and auditory deafness that develop as a result of the localized thiamin deficiency that occurs in affected tissues.[9–11] However, plasma level of thiamin in TRMA patients remains within the normal range,[12] suggesting that THTR-2 (or another as yet unidentified carrier) could provide sufficient intestinal thiamin uptake in THTR-1 deficiency. These observations were reproduced experimentally in THTR-1–deficient mice.[13,14] Our aims in this study were to examine the role of THTR-2 in intestinal thiamin uptake in a physiologically relevant setting and to test the possibility that this transporter can provide sufficient intestinal thiamin uptake in the absence of functional THTR-1. To do this, we generated a THTR-2–deficient mouse model and examined the effect of knocking out the *SLC19A3* gene (the

Abbreviations used in this paper: PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; THTR, thiamin transporter; TRMA, thiamin-responsive megaloblastic anemia.

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gene that encodes THTR-2) on intestinal thiamin uptake. We also obtained THTR-1–deficient mice[13] and investigated the impact of knocking out the *SLC19A2* gene on intestinal thiamin uptake. Our results showed intestinal thiamin uptake in the THTR-2 (but not THTR-1)–deficient mice to be significantly lower than that of wild-type littermate mice. This impairment in intestinal thiamin uptake was associated with a significant decrease in blood thiamin level in the THTR-2–deficient mice. Further, although knocking out THTR-2 is not associated with changes in the level of expression of THTR-1 in the intestine, knocking out THTR-1 is associated with a marked induction in the level of intestinal THTR-2.

Materials and Methods

Materials

[^{3}H]-Thiamin and [^{3}H]-biotin (specific activity >30 Ci/mmol; radiochemical purity >98%) were purchased from ARC (St Louis, MO). All chemicals and reagents used in this study were of analytical/molecular biology grade and were purchased from commercial sources. Cellulose nitrate filters (0.45-μm pore size) used in the uptake studies were purchased from Sartorius Filters (Hayward, CA).

Generation of THTR-2–Deficient Mice

A conventional targeting vector was constructed using a 13.9-kb region of the *THTR-2* gene sub cloned from a positively identified BAC clone (inGenious Targeting Laboratory, Inc, Stony Brook, NY). The vector was designed to allow homologous recombination to occur with a neo cassette replacing 4.2 kb of the gene including exons 1 and 2 (contains ATG start codon). Embryonic stem cells were transfected, selected, and screened then positive clones were microinjected into foster mice to produce chimera pups. Subsequent breeding with wild-type C57Bl6 mice produced F1 pups. Six heterozygous knockout mice (*Slc19a3*$^{+/-}$) were confirmed by polymerase chain reaction (PCR) and Southern blot and shipped to our facility. Inbreeding was used to produce homozygous knockout (*Slc19a3*$^{-/-}$) mice and maintain the colony. Genotyping of pups was performed using PCR screening with oligonucleotides that confirm insertion of the neo cassette (F-5′-TGCGAGGCCAGAGGCCACTTGTGTAGC-3′ and R-5′-TGACCTGATTTCCTTGAGGG-3′) as well as those that allow detection of the wild-type gene (F-5′-AGTCCTATGGGAACACAAGGCACCCTC-3′ and R-5′-TCGTGATCTTCCTGCTACAG-3′). The primer sets amplify fragments of approximately 2 and 2.1 kb in length, respectively. All mouse studies performed were reviewed and approved before animal use by the Long Beach VA Subcommittee on Animal Studies and the UCI Institutional Animal Care and Use Committee, both AAALAC-accredited institutions.

Isolation of Mouse Intestinal Epithelial Cells, In Vivo Intestinal Loop, and Uptake Studies

Intestinal epithelial cells were isolated from adult mice (2–3 mo) as described by us before,[15] using a well-established fractionation procedure.[16] We collected 5 consecutive fractions that represent mostly villus tip cells.[15] Uptake of [^{3}H]-thiamin or [^{3}H]-biotin by cells was measured as described by us previously[15] using an established rapid-filtration technique[17] at 37°C in Krebs–Ringer buffer at pH 7.4. Labeled and unlabeled vitamin was added to the incubation medium at the onset of incubation, and uptake was examined during the initial linear period of uptake (data not shown). Protein concentrations were measured using a Bio–Rad (Hercules, CA) protein determination kit. Uptake data were expressed in fmol/mg protein/10 min. Intact intestinal loops (5 cm) in vivo were prepared in the jejunal area (as described previously[18]) and filled with 250 μL of Krebs–Ringer buffer containing labeled alone or labeled plus unlabeled thiamin or biotin. Uptake was measured after 10 minutes (linear phase of uptake, data not shown). Uptake data were expressed in fmol/mg tissue wet weight/10 min. All in vitro and in vivo uptake experiments with knockout mice were run simultaneously with sex-matched wild-type littermate mice.

Establishment of THTR-1–Deficient Mice Colony

Founders of the THTR-1–deficient mouse colony were kindly provided by Dr Bruce D. Gelb (Mount Sinai School of Medicine, New York, NY).[13] Genotyping of pups was performed as described before[13] using PCR screening with oligonucleotides that confirm insertion of the neo cassette (F-5′-CTCGTCCTGCAGTTCATTCA-3′ and R-5′-AGACAATCGGCTGCTCTGAT-3′) as well as those that allow detection of the wild-type gene (F-5′-TTACCTGCTGCTGCTGTTTC-3′ and R-5′-GATGGTTAGCTGCTGGGGTA-3′). The primer sets amplify fragments of approximately 120 bp and 500 bp in length, respectively.

Quantitative Real-Time PCR

Quantitative PCR (qPCR) was performed using the Bio-Rad iCycler and a Qiagen Quantitect SYBR green PCR kit (Valencia, CA). Semiquantitative PCR was performed using the Clontech Advantage Polymerase 2 Kit (Mountain View, CA). RNA from adult (2–3 mo) mouse tissue was isolated using Trizol (Invitrogen, Carlsbad, CA) following the manufacturer's procedure. The RNA was DNase treated and first-strand complementary DNA was made from 5 μg of the isolated total RNA primed with oligo dT using an Invitrogen Superscript synthesis system. A dilution series of the reverse transcription (RT) products (1, 1/10, and 1/100) then was used in the subsequent qPCR. A 1/25 dilution of RT products was used for semiquantitative PCR. Primers used in the qPCR