Adaptive regulation of intestinal thiamin uptake: molecular mechanism using wild-type and transgenic mice carrying hTHTR-1 and -2 promoters

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Reidling, Jack C., and Hamid M. Said. Adaptive regulation of intestinal thiamin uptake: molecular mechanism using wild-type and transgenic mice carrying hTHTR-1 and -2 promoters. Am J Physiol Gastrointest Liver Physiol 288: G1127–G1134, 2005. First published February 10, 2005; doi:10.1152/ajpgi.00539.2004.—Thiamin participates in metabolic pathways contributing to normal cellular functions, growth, and development. The molecular mechanism of the human intestinal thiamin absorption process involves the thiamin transporters-1 (hTHTR-1) and -2 (hTHTR-2), products of the SLC19A2 and SLC19A3 genes. Little is known about adaptive regulation of the intestinal thiamin uptake process or the molecular mechanism(s) involved during thiamin deficiency. In these studies, we addressed these issues using wild-type mice and transgenic animals carrying the promoters of the hTHTR-1 and -2. We show that, in thiamin deficiency, a significant and specific upregulation in intestinal carrier-mediated thiamin uptake occurs and that this increase is associated with an induction in protein and mRNA levels of hTHTR-2 but not hTHTR-1; in addition, an increase in the activity of the SLC19A3 gene but not the SLC19A2 promoter was observed in the intestine of transgenic mice. Similar findings were detected in the kidney; however, expression of both thiamin transporters and activity of both human promoters were upregulated in this organ in thiamin deficiency. We also examined the effect of thiamin deficiency on the level of expression of mTHTR-1 and mTHTR-2 messages and activity of the human promoters in the heart and brain of transgenic mice and found an increase in mTHTR-1 mRNA and a rise in activity of the SLC19A2 promoter in thiamin-deficient mice. These results show that the intestinal and renal thiamin uptake processes are adaptively upregulated during dietary thiamin deficiency, that expression of mTHTR-1 and mTHTR-2 is regulated in a tissue-specific manner, and that this upregulation is mediated via transcriptional regulatory mechanism(s).

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THIAMIN, VITAMIN B1, PLAYS A fundamental role in metabolism and energy production as the coenzyme thiamin pyrophosphate. As an essential vitamin in mammals, it is required for normal cellular functions, growth, and development. Dietary thiamin deficiency leads to neurological and cardiovascular disorders (1, 41, 47), and occurs in a variety of patients, including alcoholics (8, 17, 19, 39, 40, 43, 44), diabetics (38), and those with celiac and renal diseases (24, 26, 42). Mammals obtain thiamin by absorption in the intestine via a specialized carrier-mediated mechanism (4, 5), 16, 29, 36, 37). The intestine, therefore, assumes an obviously important role in maintaining and regulating normal thiamin body homeostasis; however, the kidneys also participate in this regulation via their role in reabsorption of filtered thiamin. Similar to the situation in the intestine, renal uptake of thiamin is also through a specialized carrier-mediated mechanism (46).

The molecular characteristics of the intestinal thiamin absorption process have been elucidated after the cloning of two thiamin transporters from a number of human and mouse tissues (3, 6, 7, 10, 11, 15, 22, 25). These transporters are the thiamin transporter-1 (THTR-1; in humans it is referred to as hTHTR-1, the product of the SLC19A2 gene; in the mouse it is referred to as mTHTR-1; see Refs. 3, 6, 11, 15) and the thiamin transporter-2 (THTR-2; in humans it is referred to as hTHTR-2, the product of the SLC19A3 gene; in the mouse it is referred to as mTHTR-2; see Refs. 7 and 25). The cloned cDNAs of the human transporters were found to encode proteins of 497 and 496 amino acids, both with 12 putative trans-membrane domains. The human transport proteins share 48% amino acid identity with each other. Previous work from a number of laboratories has reported the expression patterns for hTHTR-1 and hTHTR-2 messages (3, 6, 7, 11, 15, 28, 32). In general, the hTHTR-1 is expressed at higher levels than hTHTR-2 and in a broad range of tissues (3, 6, 11, 15, 28). Studies from our laboratory have shown that both the hTHTR-1 and the hTHTR-2 are expressed in specific tissues of the human gastrointestinal tract, including the small and large intestine (28, 32). In addition, we have shown that, although the hTHTR-1 protein is expressed at both the apical and basolateral membrane domains of the polarized enterocyte, expression of the hTHTR-2 protein appears to be limited to the apical membrane domain of the cell (2). Furthermore, we have shown that both hTHTR-1 and hTHTR-2 are involved in intestinal thiamin uptake and that together they account for all the carrier-mediated uptake of the vitamin by intestinal absorptive cells (32). Moreover, we have recently cloned and characterized the promoters for the SLC19A2 and SLC19A3 genes and demonstrated their activity in vitro, and in vivo using transgenic mice (20, 27).

In contrast to our expanding knowledge about the mechanism of the intestinal thiamin uptake process and the systems involved, we know little about regulation of the process by substrate availability and about the molecular mechanism(s) involved during that type of regulation (16). Transport of a variety of nutrients is regulated by substrate availability (9, 13, 33–35). This adaptive regulation in the transport events is both substrate- and tissue-specific in nature. For example, dietary deficiencies of biotin, folate, and riboflavin lead to an upregulation in their intestinal uptake, whereas a decrease in dietary levels of glucose and amino acids lead to a downregulation in...
their intestinal uptake (9, 13, 33–35). In addition, although the intestinal absorption of the Na+-dependent phosphate cotransporter is adaptively upregulated during dietary phosphate deficiency in the intestine, no such adaptive regulation occurs in the pulmonary epithelia (45).

Our objective in these investigations was to examine the effect of dietary thiamin deficiency on intestinal thiamin absorption and to determine the molecular mechanism involved in any observed regulation. In addition, we were interested in examining the effect of thiamin deficiency on renal thiamin transport. We also tested if expression of the thiamin transporters is affected by this condition in the heart and brain (since cardiovascular and neurological disorders are typical clinical symptoms of thiamin deficiency). We chose the mouse as an animal model system since it has orthologs (Slc19a2 and Slc19a3) to the human thiamin transporters, and in our investigations we used both wild-type and transgenic mice carrying the human SLC19A2 and SLC19A3 promoters. The use of transgenic mice in these studies was to assist us in determining if transcriptional mechanisms are involved in any regulation of thiamin uptake during thiamin deficiency. The use of such animals may also allow us to determine, in an in vivo setting, the possible effect of dietary thiamin deficiency on activity of the human SLC19A2 and SLC19A3 promoters. The results of our investigations indicate that the intestinal thiamin uptake process is adaptively upregulated during dietary thiamin deficiency and that the upregulation appears to be mediated via transcriptional regulatory mechanism(s) involving THTR-2, but not THTR-1. Similar findings were observed in the kidney; however, the molecular mechanism(s) appears to involve both THTR-1 and THTR-2. On the other hand, a predominant upregulation in the level of expression of the THTR-1 system occurs in the heart and brain during thiamin deficiency. Taken together, the findings also show that regulation of the thiamin transporters during thiamin deficiency is tissue specific in nature.

MATERIALS AND METHODS

Materials

[1H]Thiamin and [1H]biotin (sp act >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 vesicle uptake studies, were purchased from Sartorius Filters (Hayward, CA).

Induction of Dietary Thiamin Deficiency in Mice

Wild-type and transgenic mice [previously generated for us by the University of California, Irvine (UCI) Transgenic Mouse Facility (TMF); see Refs. 20 and 27] were pair-fed either a thiamin-deficient or a control diet (the latter diet is the same as the deficient diet but with added thiamin, 6 mg/kg; Dyets, Bethlehem, PA) and tap water ad libitum for 3–4 wk, as described previously (23). Thiamin deficiency was confirmed by testing (LabCorp, Research Triangle Park, NC) serum thiamin levels at the time of death, which was determined to be 7.3 ± 1.5 and 228 ± 44 (P < 0.01) mg/dl for thiamin-deficient and pair-fed control mice, respectively. Mice were killed by CO2 inhalation and cervical dislocation, followed by immediate tissue collection. The proximal small intestine (mucosal scrapings), kidney, heart, and the brain were collected in either ice-cold TRIZol (Invitrogen, Carlsbad, CA) for RNA isolation, Passive Lysis Buffer (Promega, Madison, WI) for luciferase assays, snap-frozen in liquid nitrogen for protein analysis, or used for the isolation of brush-border membrane vesicles (BBMV; intestinal mucosa and kidney only), as described below. All mouse studies performed were reviewed and approved before animal use by the Long Beach Veterans Affairs Subcommittee on Animal Studies and the University of California Irvine Institutional Animal Care and Use Committee, both Association for the Assessment and Accreditation of Laboratory Animal Care-accredited institutions.

Preparation of Intestinal and Renal BBMV and Transport Studies

Intestinal and renal BBMV were prepared as described by us previously (21) using a modification of the Kessler’s divalent cation (Mg2+) precipitation technique (14). All isolation steps were carried out at 4°C. Transport studies were performed using freshly prepared vesicles and a rapid filtration technique (12) as shown in our previous work (21). All uptake studies were performed at 37°C for 10 s (initial rate; data not shown). Protein concentrations were measured using a Bio-Rad (Hercules, CA) protein determination kit.

Quantitative Real-time PCR and Western Blot Analysis

Quantitative real-time PCR (qPCR) was performed using the Bio-Rad iCycler and a Qiagen Quantitect SYBR Green PCR kit (Valencia, CA). RNA from intestinal mucosa scrapings, kidney, heart, or brain was isolated using TRIZol (Invitrogen) and the manufacturer’s procedure. The RNA was DNase treated, and first-strand cDNA was made from 5 μg of the isolated total RNA primed with oligo(dT) using an Invitrogen Super-script synthesis system. A dilution series of the RT products (1, 1:10, and 1:100) was then used in the subsequent qPCR. Primers used in the qPCR were specific for the mouse Slc19a2 (forward: 5’-GGTCTCTACGCTTACCTTC-3’, reverse: 5’-GCATGAAACCACGTCAACATC-3’), the mouse Slc19a3 (forward: 5’-CATGCAAAACACGTGATTCT-3’, reverse: 5’-ACTCAGCTAGCCTGCTCA-3’), and the mouse β-actin (forward: 5’-AGGCCAACGCTGTCCTGTTGA-3’, reverse: 5’-TAGAGAGGGCCCCACCC-3’). The qPCR consisted of a 15-s 95°C melt followed by 40 cycles of 95°C melt for 30 s, 58°C annealing for 30 s, and 72°C extension and data collection for 1 min. Melt curve analysis with plasmid DNA was performed for the generation of standard curves, and negative controls without RT were used with every reaction. To compare the relative relationship between Slc19a2 and Slc19a3 in deficient and pair-fed mice, we used a calculation method provided by the iCycler manufacturer (Bio-Rad) described in the legend for Fig. 2. Western blot analysis was performed as previously described (28, 32) using antibodies designed to be specific for either mTHTR-1 or mTHTR-2 proteins (Sigma-Genosys, The Woodlands, TX). Densities of specific bands were determined (as unitless measurements) using the UN-SCAN-IT gel automated digitizing system version 3.1 (Silk Scientific).

Human SLC19A2 and SLC19A3 Promoter Activity in Transgenic Mice

Thiamin-deficient and pair-fed transgenic mice expressing the 320-bp SLC19A2 or 2,016-bp SLC19A3 promoters fused to the firefly luciferase reporter gene were generated for us by the UCI-TMF and have been previously characterized in our laboratory (20, 27). Mice were killed, and intestinal mucosa scrapings, kidney, heart, or brain were immediately removed and placed in ice-cold Passive Lysis Buffer (Promega) for luciferase assays. The tissue was immediately lysed on ice using a PowerGen125 (Fisher, Pittsburgh, PA) hand blender and frozen at −80°C. Luciferase assays were performed using the manufacturer’s procedures and a Turner Design TD-20/20 luminometer. Luciferase assays were normalized to total protein for each sample measured using the DC protein assay kit (Bio-Rad).

Statistical Analysis

Transport data presented in this paper are the result of three separate experiments and are expressed as means ± SE in ficmole
Consequence of thiamin deficiency on the level of expression of the mTHTR-1 and mTHTR-2 protein and mRNA in the intestine. To obtain information on the molecular mechanisms involved in the adaptive regulation of the intestinal thiamin uptake process, we examined the effect of thiamin deficiency on the protein levels of mTHTR-1 and mTHTR-2 using Western blot analysis and specific polyclonal antibodies against the mouse transporters. The results (Fig. 2A) showed the levels of the mTHTR-2 protein to be significantly \((P < 0.01)\) higher in the small intestine of thiamin-deficient mice compared with control mice (densities of the specific bands were \(277 \pm 0.8\) and \(12 \pm 0.4\), respectively). On the other hand, no increase in the level of mTHTR-1 protein was observed in the two mice groups, rather a slight decrease was noticed (densities of the specific bands were \(207 \pm 0.7\) and \(197 \pm 0.5\), for deficient and control mice, respectively; Fig. 2A).

In another study, we examined the effect of thiamin deficiency on mRNA levels of mTHTR-1 and mTHTR-2 using qPCR and gene-specific primers. The results (Fig. 2B) showed the level of mTHTR-2 mRNA to be significantly \((P < 0.01)\) higher in the small intestine of the thiamin-deficient mice compared with controls. However, no such increase in the level of mTHTR-1 mRNA was observed in thiamin-deficient mice compared with controls (rather a slight but not significant decrease was noticed; Fig. 2B).

The above results indicate that the increase in intestinal thiamin uptake in thiamin-deficient mice is mediated via an increase in the level of mTHTR-2 protein and mRNA levels and points toward the possible involvement of transcriptional regulatory mechanism(s).

Effect of thiamin deficiency on activity of the human SLC19A2 and SLC19A3 promoters expressed in vivo in the intestine of transgenic mice. To assess the possibility that the increase in intestinal thiamin uptake and the level of expression of the thiamin transporter messages are the result of transcriptional regulatory events, we used transgenic mice carrying the human SLC19A2 and SLC19A3 promoters attached to luciferase reporter genes \((20, 27)\). The results showed that, in the intestine of mice with the SLC19A3 promoter-luciferase transgene, activity of the SLC19A3 promoter was significantly \((P < 0.01)\) higher during a thiamin-deficient condition compared with controls (Fig. 3). On the other hand, activity of the SLC19A2 promoter in the intestine of mice with the SLC19A2 promoter-luciferase transgene did not increase during thiamin deficiency.

**RESULTS**

**Effect of Dietary Thiamin Deficiency on Intestinal Thiamin Uptake and the Molecular Parameters of the Uptake Process**

Outcome of thiamin deficiency on intestinal carrier-mediated thiamin uptake. In this study, we examined the effect of dietary thiamin deficiency on intestinal thiamin uptake using BBMV isolated from the proximal part of the small intestine of thiamin-deficient and pair-fed control mice. The results showed the carrier-mediated uptake of a physiological concentration of \(^{3}H\)thiamin \((15 \text{ nM})\) to be significantly \((P < 0.01)\) higher in thiamin-deficient mice compared with pair-fed controls (Fig. 1). In contrast, carrier-mediated uptake of the unrelabeled \(^{3}H\)biotin \((9.6 \text{ nM})\) was similar in the two mice groups (Fig. 1). These findings establish that carrier-mediated intestinal thiamin uptake is adaptively upregulated at the functional level during thiamin deficiency and shows that the regulation is specific in nature.

**Fig. 1.** Effect of thiamin deficiency on carrier-mediated thiamin uptake by intestinal brush-border membrane vesicles (BBMV) isolated from wild-type mice. Initial rate of carrier-mediated uptake of a physiological concentration of thiamin \((15 \text{ nM})\) or biotin \((9.6 \text{ nM})\) by purified intestinal BBMV isolated from thiamin-deficient and control pair-fed mice was determined as described in MATERIALS AND METHODS. Each data point represents the mean \(\pm\) SE of 3–6 uptake determinations from different BBMV preparations isolated on separate occasions from 3–6 mice.

Consequence of thiamin deficiency on the level of expression of the mTHTR-1 and mTHTR-2 protein and mRNA in the intestine. To obtain information on the molecular mechanisms involved in the adaptive regulation of the intestinal thiamin uptake process, we examined the effect of thiamin deficiency on the protein levels of mTHTR-1 and mTHTR-2 using Western blot analysis and specific polyclonal antibodies against the mouse transporters. The results (Fig. 2A) showed the levels of the mTHTR-2 protein to be significantly \((P < 0.01)\) higher in the small intestine of thiamin-deficient mice compared with control mice (densities of the specific bands were \(277 \pm 0.8\) and \(12 \pm 0.4\), respectively). On the other hand, no increase in the level of mTHTR-1 protein was observed in the two mice groups, rather a slight decrease was noticed (densities of the specific bands were \(207 \pm 0.7\) and \(197 \pm 0.5\), for deficient and control mice, respectively; Fig. 2A).

In another study, we examined the effect of thiamin deficiency on mRNA levels of mTHTR-1 and mTHTR-2 using qPCR and gene-specific primers. The results (Fig. 2B) showed the level of mTHTR-2 mRNA to be significantly \((P < 0.01)\) higher in the small intestine of the thiamin-deficient mice compared with controls. However, no such increase in the level of mTHTR-1 mRNA was observed in thiamin-deficient mice compared with controls (rather a slight but not significant decrease was noticed; Fig. 2B).

The above results indicate that the increase in intestinal thiamin uptake in thiamin-deficient mice is mediated via an increase in the level of mTHTR-2 protein and mRNA levels and points toward the possible involvement of transcriptional regulatory mechanism(s).

**Effect of thiamin deficiency on activity of the human SLC19A2 and SLC19A3 promoters expressed in vivo in the intestine of transgenic mice.** To assess the possibility that the increase in intestinal thiamin uptake and the level of expression of the thiamin transporter messages are the result of transcriptional regulatory events, we used transgenic mice carrying the human SLC19A2 and SLC19A3 promoters attached to luciferase reporter genes \((20, 27)\). The results showed that, in the intestine of mice with the SLC19A3 promoter-luciferase transgene, activity of the SLC19A3 promoter was significantly \((P < 0.01)\) higher during a thiamin-deficient condition compared with controls (Fig. 3). On the other hand, activity of the SLC19A2 promoter in the intestine of mice with the SLC19A2 promoter-luciferase transgene did not increase during thiamin deficiency.

**Fig. 2.** Effect of dietary thiamin deficiency on the level of expression of the mouse (m) thiamin transporter (THTR)-1 and mTHTR-2 expression in mouse intestinal tissue. A: Western blot analysis was performed using 150 \(\mu\)g membrane protein fraction from either thiamin-deficient or pair-fed control mouse intestinal mucosa and probed with polyclonal antibodies specific for mTHTR-1 or mTHTR-2. The bound antibodies were detected using the ECL system. The results showed are a representative experiment from a pool of 3 independent sets. B: quantitative real-time PCR (qPCR) was performed using gene-specific primers and total RNA isolated from the small intestine of thiamin-deficient or pair-fed control mice. Data are from at least 3 mice with qPCR performed three times and were normalized relative to \(\beta\)-actin and calculated using a relative relationship method supplied by the iCycler manufacturer (Bio-Rad). Data are shown as means \(\pm\) SE and are presented as expression over mTHTR-2 control, which was set at 1.
deficiency compared with controls, rather a slight decrease was observed (Fig. 3). These results suggest that the adaptive regulation of the intestinal thiamin uptake process in thiamin-deficient mice may involve transcriptional regulatory mechanisms for the SLC19A3 promoter. The results also show that the promoters of the human thiamin transporters behave in the intestine in vivo in a parallel fashion to the endogenous mouse thiamin transporters. In addition, the results may imply that thiamin deficiency in humans could similarly affect the activity of the SLC19A3 promoter in the human intestine.

Effect of Dietary Thiamin Deficiency on Renal Thiamin Uptake and the Molecular Parameters of the Uptake Process

Outcome of thiamin deficiency on renal carrier-mediated thiamin uptake. The effect of dietary thiamin deficiency on renal thiamin carrier-mediated uptake in wild-type mice was examined using BBMV isolated from the kidney cortex of thiamin-deficient and pair-fed control mice. The results showed that the carrier-mediated uptake of a physiological concentration of [3H]thiamin (15 nM) was significantly (P < 0.01) higher in thiamin-deficient mice compared with pair-fed controls (Fig. 4). In contrast, the carrier-mediated uptake of the unrelated [3H]biotin (9.6 nM) was similar in the two mice groups (Fig. 4). These findings show that the renal thiamin uptake process is also upregulated at the functional level during thiamin deficiency and that the regulation is specific in nature.

Consequence of thiamin deficiency on the level of expression of mTHTR-1 and mTHTR-2 protein and mRNA in the kidney. The effect of thiamin deficiency on the level of expression of the mTHTR-1 and mTHTR-2 protein in the kidney was examined using Western blot analysis, as described earlier. The results (Fig. 5A) show a significant (P < 0.01 for both) increase in protein levels for both thiamin transporters in thiamin-deficient mice compared with controls (densities of the specific bands were 335 ± 0.3 and 130 ± 0.4 for mTHTR-1 and 296 ± 0.7 and 199 ± 0.6 for mTHTR-2, respectively).

We also examined the effect of dietary thiamin deficiency on the level of expression of mTHTR-1 and mTHTR-2 mRNA in the kidney using qPCR. The results (Fig. 5B) showed a significant (P < 0.01 for both) increase in the level of message expression for both of the thiamin transporters in the thiamin-deficient condition compared with controls. These results indicate that the increase in renal thiamin uptake in thiamin-deficient mice is mediated via an increase in the level of mTHTR-1 and mTHTR-2 protein and mRNA levels and again points toward the possible involvement of transcriptional regulatory events.

Effect of thiamin deficiency on activity of the human SLC19A2 and SLC19A3 promoters expressed in vivo in the kidneys of transgenic mice. We evaluated the likelihood that, as in the intestine, the increase in renal thiamin uptake and the level of expression of the thiamin transporter messages in thiamin deficiency are the result of transcriptional regulatory events. This was performed using transgenic mice carrying the human SLC19A2 and SLC19A3 promoters attached to luciferase-reporter genes. The results (Fig. 6) showed a significantly (P < 0.01 for both) higher activity of SLC19A2 and SLC19A3 promoters in the kidney of transgenic mice made thiamin deficient compared with controls (Fig. 6). Our findings suggest that the adaptive regulation in renal thiamin uptake may involve transcriptional regulatory events and that the promoters of the human thiamin transporters behave in the kidney in vivo in a parallel fashion to the endogenous mouse thiamin transporters. The results may also imply that thiamin deficiency in humans could similarly affect the activity of the SLC19A2 and SLC19A3 promoters in the human kidneys.

Effect of dietary thiamin deficiency on molecular parameters of the thiamin transporters in the heart and the brain. We examined the consequence of dietary thiamin deficiency on the levels of expression of mTHTR-1 and mTHTR-2 mRNA in two of the tissues that are known to exhibit clinical symptoms during thiamin deficiency, namely the heart and brain. The results showed that mTHTR-1 mRNA was significantly (P < 0.01) increased in both the heart and the brain in thiamin-deficient mice compared with controls (Fig. 7, A and B). On the other hand, only a minor increase in mTHTR-2 message level was observed in the heart, and no increase was detected in the brain of the thiamin-deficient mice compared with controls (Fig. 7, A and B).

In other studies, we examined the effect of dietary thiamin deficiency on the activity of the SLC19A2 and SLC19A3 promoters in the heart and brain of transgenic mice expressing
the human promoter-luciferase reporter genes and compared the findings with those obtained with transgenic mice pair-fed the control diet. The results showed a predominant increase in the activity of the SLC19A2 promoter in both tissues and only a minor increase in the activity of the SLC19A3 promoter in the thiamin-deficient mice compared with controls (Fig. 8, A and B).

**DISCUSSION**

The objective of the current study was to examine the possibility that adaptive regulation occurs in the intestinal thiamin uptake process in thiamin deficiency and to determine the molecular mechanism(s) involved in any observed regulation. We were also interested in examining the effect of thiamin deficiency on renal transport of thiamin. In addition, the level of expression of the THTR-1 and THTR-2 in the heart and the brain was also determined in these studies. The latter two tissues are highly susceptible to the effect of thiamin deficiency, as evidenced by the nature of clinical symptoms that arise from dietary deficiency of this vitamin. We used the mouse as an animal model in our investigations given that this species has orthologs for the two recently identified human thiamin transporters and because transgenic mice that carry the species has orthologs for the two recently identified human species was the target of the increased expression of mTHTR-2. The results also point toward the possible involvement of transcriptional regulatory mechanisms in the promoter for the THTR-2 system as mediating the upregulatory events. To test the latter possibility, we took advantage of the availability in our laboratory of transgenic mice carrying the promoters for the human thiamin transporters SLC19A2 and SLC19A3. Studies using these transgenic mice showed that thiamin deficiency was associated with an increase in the activity of the human SLC19A3 (but not the SLC19A2) promoter in vivo in the small intestine. These findings support the notion that transcriptional mechanism(s), via the THTR-2 system, may be involved in the regulation of the intestinal thiamin absorption process. In addition, these data may imply that dietary thiamin deficiency may similarly affect the activity of the SLC19A3 promoter in vivo in the human intestine. One possible explanation for the specific increase in THTR-2 levels over THTR-1 may be that the THTR-2 system is more sensitive to regulatory mechanisms in the intestine while THTR-1 provides basal activity. Our findings in the studies described above have relevance to the clinical condition of thiamin-responsive megaloblastic anemia (TRMA). TRMA is an autosomal recessive disorder characterized by manifestations of megaloblastic anemia, sensorineural deafness, and diabetes mellitus. The cause of TRMA is believed to be mutations in the THTR-1 that lead to the development of a localized thiamin deficiency in the affected tissues. Interestingly, patients with this disorder have normal blood thiamin levels, permitting one to speculate that an alternative thiamin uptake system compensates for the loss of function of THTR-1, thus allowing for maintenance of intestinal thiamin absorption. Our results may support this idea by showing that mTHTR-2 levels are specifically upregulated...
during a thiamin-deficient state, an event that leads to an increase in the capacity of the intestine to absorb thiamin. Our work also investigated the possible adaptive regulation of the renal thiamin transport process in thiamin deficiency. Renal uptake of thiamin also occurs by a specialized carrier-mediated mechanism (46 and unpublished observations from our laboratory), and the thiamin transporters THTR-1 and THTR-2 are expressed in the kidney. The results showed that dietary thiamin deficiency leads to a specific and significant upregulation in carrier-mediated renal thiamin uptake compared with controls that was associated with an increase in protein and mRNA levels for both the mTHTR-1 and mTHTR-2 and an increase in the activity of the SLC19A2 and SLC19A3 promoters. These results show that the renal thiamin uptake process is adaptively upregulated during thiamin deficiency and that this upregulation involves both the mTHTR-1 and mTHTR-2. In addition, the data suggest the possible involvement of transcriptional regulatory mechanisms in the upregulatory event and imply that the adaptive regulation observed in the SLC19A2 and SLC19A3 promoters in vivo may also occur in the human kidneys. However, we should note that our results do not rule out the possibility that the observed increase in transporter protein levels and uptake may also be the result of some posttranscriptional modifications. Taken together with the data obtained from the intestine, the results suggest that the adaptive regulation of the thiamin transport systems is tissue specific in nature. A reasonable justification for the increase in both transporters in the kidney during thiamin deficiency is that, under a thiamin-deficient state, reabsorption of filtered thiamin in the kidneys may become critical to the survival of the animal, and thus very efficient uptake is needed to salvage the scarce amounts of vitamin and prevent its loss. The observation of a tissue-specific regulation of the transport process is a novel discovery for thiamin; however, it has also been described for other transporters, as mentioned earlier (45).

To complement our work, we examined the effect of dietary thiamin deficiency on expression of the thiamin transporters in two tissues where severe dietary thiamin deficiency manifests as cardiovascular and neurological disorders, i.e., the heart and the brain. Interestingly, mTHTR-1 appears to be adaptively regulated in both the heart and brain using the mRNA or human promoter-reporter expression as a gauge to measure regulation. The story is not as clear with mTHTR-2 in that there is a minor increase in mouse endogenous mRNA levels with a corresponding increase in promoter activity in the heart. However, no increase was observed in endogenous mouse mRNA levels in the brain, yet there was an increase in promoter activity. One possibility is that this may be where the two species differ in the regulation of gene activity. We have observed this phenomenon previously with SLC19A2 promoter-luciferase transgenic mice (27).

In conclusion, results of these studies show that both the intestinal and the renal thiamin uptake processes are adaptively regulated during dietary thiamin deficiency and that transcriptional mechanisms may be involved in this regulation. In addition, dietary thiamin deficiency appears to regulate expression of the thiamin transporters in a tissue-specific manner. Further studies providing additional information on the exact nature of the transcriptional regulatory events (including the identity of the transcription factors...
involved) are, however, needed and may be the subject of future investigations.

GRANTS

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