Chronic alcohol consumption and intestinal thiamin absorption: effects on physiological and molecular parameters of the uptake process

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Subramanya SB, Subramanian VS, Said HM. Chronic alcohol consumption and intestinal thiamin absorption: effects on physiological and molecular parameters of the uptake process. Am J Physiol Gastrointest Liver Physiol 299: G23–G31, 2010. First published May 6, 2010; doi:10.1152/ajpgi.00132.2010.—Thiamin is essential for normal cellular functions, and its deficiency leads to a variety of clinical abnormalities. Humans and other mammals obtain the vitamin via intestinal absorption. The intestine is exposed to two sources of thiamin, a dietary and a bacterial (i.e., normal microflora of the large intestine) source. Chronic alcohol consumption is associated with thiamin deficiency, which is caused (in part) by inhibition in intestinal thiamin absorption. However, little is known about the physiological and molecular aspects of the intestinal thiamin uptake process that are affected by chronic alcohol use. To address these issues, we used rats fed an alcohol-liquid diet and human intestinal epithelial HuTu-80 cells chronically exposed to ethanol as model systems. The results showed that chronic alcohol feeding to rats led to a significant inhibition in carrier-mediated thiamin transport across both the jejunal brush-border membrane and basolateral membrane domains. This was associated with a significant reduction in level of expression of thiamin transporter-1 (THTR-1), but not THTR-2, at the protein and mRNA levels. Level of expression of the heterogenous nuclear RNA of THTR-1 in the intestine of alcohol-fed rats was also decreased compared with their pair-fed controls. Chronic alcohol feeding also caused a significant inhibition in carrier-mediated thiamin uptake in rat colon. Studies with HuTu-80 cells chronically exposed to ethanol also showed a significant inhibition in carrier-mediated thiamin uptake. This inhibition was associated with a reduction in level of expression of human THTR-1 and THTR-2 at the protein, mRNA, and transcriptional (promoter activity) levels. These studies demonstrate that chronic alcohol feeding inhibits intestinal thiamin absorption via inhibition of the individual membrane transport event across the polarized absorptive epithelial cells. Furthermore, the inhibition is, at least in part, mediated via transcriptional mechanism(s).

vitamin B1; brush-border membrane vesicles; basolateral membrane vesicles; thiamin transporter-1; thiamin transporter-2

Thiamin (vitamin B1), a water-soluble micronutrient, plays an essential role in normal cellular functions, growth, and development. The vitamin acts as a cofactor for multiple enzymes (transketolase, pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and branched chain ketoacid dehydrogenase) that are involved in a variety of critical metabolic reactions related to energy metabolism (5). Furthermore, because thiamin bridges the glycolytic pathway and the pentose phosphate metabolic pathway, which is a critical step in creating chemical-reducing power in cells, this micronutrient also plays a role in reducing cellular oxidative stress (15). Thiamin deficiency in humans leads to a variety of abnormalities including neurological and cardiovascular disorders (3, 41, 45). On the other hand, optimization of thiamin status may prevent diabetic retinopathy and block tissue damage caused by hyperglycemia of diabetes (14). Thiamin deficiency and suboptimal status represent significant nutritional problems worldwide and are associated with a variety of conditions including chronic alcohol consumption (17, 41–43, 45).

Humans (and all other mammals) cannot synthesize thiamin and thus must obtain the vitamin from exogenous sources via intestinal absorption. Therefore, the intestine plays a central role in regulating body homeostasis of the vitamin. The human intestine mediates absorption from two sources of thiamin: a dietary thiamin (which is absorbed in the small intestine) and a bacterial thiamin (where the vitamin is produced in significant quantities by the normal microflora of the large intestine and is absorbed primarily in that region of the gut). In both the small and large intestine, absorption of thiamin occurs via an efficient and specialized carrier-mediated processes (reviewed in Refs. 32 and 36). Thiamin was found at both membrane domains of the polarized intestinal absorptive cells, i.e., across the intestinal brush-border membrane (BBM) and basolateral membrane (BLM) domains (reviewed in Refs. 32 and 36). Studies from our laboratory have also shown that both thiamin transporter-1 and -2 (THTR-1 and THTR-2), products of the SLC19A2 and SLC19A3 genes, respectively, are involved in intestinal thiamin uptake (27, 33). Furthermore, we have also shown that, whereas THTR-1 is expressed at the both the apical BBM and BLM domains of the intestinal epithelial cells,
expression of THTR-2 is restricted to the apical BBM domain only (33, 39).

Chronic alcohol consumption leads to thiamin deficiency, and that inhibition in intestinal thiamin absorption process plays a role in causing this abnormality (9, 17). However, little is known about the physiological and molecular aspects of the intestinal thiamin absorption process that are affected by chronic alcohol consumption. In this study, we investigated the effect of chronic alcohol use on both transport across the small intestinal BBM and/or BLM, whether one or both of the thiamin transport systems (i.e., THTR-1 and THTR-2) are affected, and the mechanism(s) that mediates the effect. We also investigated whether thiamin absorption in the colon is affected by chronic alcohol feeding. We used two models in our investigations: an in vivo model of chronic alcohol feeding affected by chronic alcohol feeding. We used two models in our investigations: an in vivo model of chronic alcohol feeding affected by chronic alcohol feeding.
Fig. 2. Effect of chronic alcohol feeding of rats on carrier-mediated thiamin uptake by jejunal basolateral membrane vesicles (BLMV). Carrier-mediated \(^3\)H-thiamin (0.25 \(\mu\)M) uptake by jejunal BLMV of rats fed alcohol liquid diet for 4 wk compared with their pair-fed controls. Data are means \pm SE of at least 3 separate uptake determinations from multiple sets of rats. *P < 0.01.

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then performed using the following hnRNA gene-specific primers: THTR-1 (forward, 5'-CACITTGTACCTGTGTGTC-3'; reverse, 5'-GAAGTACAGGCTTGAAC-3'); THTR-2 (forward, 5'-CTACCCGTAACAGGACACAG-3'; reverse, 5'-TGGTAAAATGGGCTTCAC-3'); and β-actin (forward, 5'-CTGCTTTTCCAGATGAG-3'; reverse, 5'-CTCTATAGTGCCACAGT-3'). A negative control, which constituted a reaction without cDNA template, was run with every assay to establish specificity. The amplified products were then run on 1% agarose gel, and the specific bands were quantified using UN-SCAN-IT gel automated digitizing system, version 6.1 (Silk Scientific, Orem, UT).

 Culturing of HuTu-80 cells and chronic alcohol exposure. The human-derived intestinal (duodenal) epithelial HuTu-80 cells (ATCC, Manassas, VA; passages 10 to 18) were plated at a density of 2 x 10^5 cells/well onto 12-well plates in MEM media (ATCC) supplemented with 10% FBS and antibiotics. Twenty-four hours later, fresh growth medium containing 200 mM alcohol (a concentration that simulates the level of alcohol encountered by the intestine in chronic alcohol drinkers; Ref. 26) was introduced, and the plates were kept in an ethanol-saturated incubator for 72 h as described recently with minor modifications (46); in addition to changing the alcohol-containing growth medium every 12 h, studies were designed to minimize ethanol evaporation (46). Uptake studies were performed at 37°C in Krebs-Ringer buffer (in mM: 133 NaCl, 4.93 KCl, 1.23 MgSO4, 0.85 CaCl2, 5 glucose, 5 glutamine, 10 HEPES, 10 MES, pH 7.4). Labeled and unlabeled thiamin were added to the incubation medium at the onset of incubation, and uptake was examined during the initial linear period of uptake (5 min; data not shown). The reaction was terminated by the addition of 2 ml of ice-cold Krebs-Ringer buffer followed by immediate aspiration. Cells were then rinsed twice with ice-cold buffer, digested with 1 ml of 1 N NaOH, neutralized with 10 N HCl, and then measured for radioactive content using scintillation counter. Cell digest samples (100 μl) were taken for protein quantification using a Bio-Rad Dc protein assay kit.

Transfection and reporter gene assay. SLC19A2 and SLC19A3 full-length promoter-luciferase reporter constructs utilized in this study were generated previously (24, 29). HuTu-80 cells were cotransfected in 12-well plates at less than 80% confluency with 4 μg of each test construct and 100 ng of the Renilla transfection control plasmid Renilla luciferase-thymidine kinase (pRL-TK) (Promega, Madison, WI). Transfection was performed with Lipofectamine reagent (Invitrogen) according to manufacturer’s instructions. On the second posttransfection day the cells transfected with SLC19A2 and SLC19A3 were treated with MEM medium containing 200 mM alcohol for 72 h. To ensure proper alcohol effect, the media containing 200 mM alcohol was changed every 12 h. At the end of 72 h of alcohol incubation, Renilla-normalized firefly luciferase activity was determined by using the Dual Luciferase Assay system (Promega).

Statistical analysis. Uptake data with rat intestinal preparation presented in this paper are means ± SE of at least three separate experiments from different rats and are expressed in femtomoles per milligram of protein per unit of time. Uptakes with the human intestinal epithelial HuTu-80 cells were also means ± SE from at least three separate experiments. Student’s t-test was used for statistical analysis. P < 0.05 was considered statistically significant. Carrier-mediated thiamin uptake was determined by subtracting uptake by simple diffusion (determined from the slope of the line between uptake at high pharmacological concentration of 1 mM and point of origin) from total uptake. Protein and RNA determinations as well as promoter assay were run on at least three different occasions using three different preparations.

RESULTS

Effect of chronic alcohol feeding of rats on thiamin transport across jejunal BBM and BLM domains: studies with isolated membrane vesicles. The effect of chronic alcohol feeding of rats for different periods of time (2, 4, and 6 wk) on the entry of a physiological concentration of thiamin (0.25 μM), i.e., transport across the apical BBM of the polarized intestinal epithelial cells, was examined using purified jejunal BBMV. Vesicles were isolated simultaneously from alcohol-fed rats and their pair-fed controls and were used for uptake studies on the day of isolation (see MATERIALS AND METHODS). The results showed a significant (P < 0.01 for all) inhibition in carrier-mediated thiamin uptake in intestinal BBMV of chronic alcohol-fed rats compared with their pair-fed controls with the inhibition being evident in 2 wk after the initiation of the alcohol feeding regimen (Fig. 1, A–C).

We also examined the effect of chronic alcohol feeding (for 4 wk; standard feeding period used in all subsequent studies) on the exit of thiamin out of the polarized enterocytes, i.e., transport across the BLM, using isolated jejunal BLMV. The
results again showed a significant ($P < 0.05$) inhibition in carrier-mediated thiamin uptake in the chronic alcohol-fed rats compared with their pair-fed controls (Fig. 2).

Effect of chronic alcohol feeding on level of expression of THTR-1 and THTR-2 proteins at the jejunal BBM and BLM domains. In this study, we examined the effect of chronic alcohol feeding on the level of expression of THTR-1 and THTR-2 proteins at the BBM and BLM domains of rat jejunum. This was performed by Western blot analysis using polyclonal antibodies against the THTR-1 and THTR-2 and purified BBM and BLM preparations isolated from the jejunum of rats fed alcohol chronically and their pair-fed controls. Specificity of the rat antibodies was established by treating with antigenic peptides (antigenic peptide sequence is described in MATERIALS AND METHODS). The results showed a significant ($P < 0.05$) decrease in the level of expression of THTR-1 (Fig. 3A) and THTR-2 (Fig. 3B) in rats fed alcohol chronically compared with their pair-fed controls.

With regard to the effect of chronic alcohol consumption on the level of expression of thiamin transporters at the BLM domain of the polarized enterocytes, the results again showed a significant ($P < 0.05$) reduction in the level of expression of the THTR-1 in alcohol-fed rats compared with their pair-fed controls (Fig. 4A). As previously recognized with human intestinal epithelial cells (33, 39), no expression for THTR-2 was detected in BLM preparations of rat jejunum (Fig. 4B).

Effect of chronic alcohol feeding of rats on expression of THTR-1 and THTR-2 at the mRNA and hnRNA levels. In this study, we used qPCR to examine the effect of chronic alcohol feeding on the level of expression of THTR-1 and THTR-2 mRNA using total RNA isolated from the jejunum of rats fed alcohol chronically and their pair-fed controls. The results showed a significant ($P < 0.01$) reduction in the level of expression of THTR-1 mRNA (Fig. 5A), but not that of THTR-2 (Fig. 5B), in rats fed alcohol chronically compared with their pair-fed controls.

To further understand the cause of reduction in mRNA level of THTR-1, we investigated the effect of chronic alcohol feeding on level of expression of the hnRNA of THTR-1. Level of hnRNA has been used as an index of transcriptional activity of different genes under different conditions (2, 8, 19). These results showed a significant ($P < 0.02$) reduction in THTR-1 hnRNA levels in the jejunum of rats fed alcohol chronically compared with their pair-fed controls (0.69 ± 0.3 and 0.95 ± 0.1 for alcohol-fed and pair-fed controls, respectively, in arbitrary units).

Effect of chronic alcohol feeding of rats on colonic uptake of thiamin. In this study we examined the effect of chronic alcohol feeding of rats on carrier-mediated uptake of a physiological concentration of thiamin (25 nM) using an established colonic sheet procedure as described by us previously (10). Chronic ethanol feeding caused an ~80% decrease in carrier-mediated thiamin uptake by colonic mucosa ($P < 0.03$, Fig. 6).

Effect of chronic alcohol exposure on physiological and molecular parameters of thiamin uptake by the human-derived intestinal epithelial HuTu-80 cells. In these investigations, we extended our studies into the human-derived intestinal epithelial HuTu-80 cells and examined the effect of chronic alcohol exposure on physiological and molecular parameters of thiamin uptake by the human-derived intestinal epithelial HuTu-80 cells.
min uptake by these cells. Chronic alcohol exposure was performed for 72 h as described recently (46). A concentration of ethanol of 200 mM was chosen to mimic the level of ethanol encountered by the human intestine following alcohol ingestion (26). The results showed a significant \( P < 0.05 \) inhibition in carrier-mediated \(^3\)H-thiamin uptake in alcohol-exposed cells compared with controls (Fig. 7A). This was associated with a significant \( P < 0.05 \) reduction in the level of expression.

Fig. 7. Effect of chronic alcohol exposure of the human-derived intestinal epithelial HuTu-80 cells on carrier-mediated thiamin uptake and level of expression of THTR-1 and THTR-2 at the protein and mRNA levels. HuTu-80 cells were chronically exposed (72 h) to 200 mM ethanol as described in MATERIALS AND METHODS. A: carrier-mediated \(^3\)H-thiamin (25 nM) uptake was carried out and compared with control. B and C: Western blot analysis of membranous fraction of HuTu-80 cells run as in Fig. 3. Data are means ± SE of at least 3 separate sets of experiments that were normalized relative to \( \beta \)-actin protein expression. \(^*\)\( P < 0.05 \). D and E: real-time quantitative PCR was performed using hTHTR-1 and hTHTR-2 gene-specific primers (see MATERIALS AND METHODS). Data are means ± SE of at least 3 separate sets of experiments and were normalized relative to \( \beta \)-actin and calculated using relative relationship method supplied by manufacturer (Bio-Rad). \(^*\)\( P < 0.05 \).
sion of hTHTR-1 and hTHTR-2 proteins (Fig. 7, B and C) and for all mRNA levels (Fig. 7, D and E).

We also examined the effect of chronic alcohol exposure of the human intestinal epithelial HuTu-80 cells on the activity of full-length SLC19A2 (−2,250 to −36 bp) and SLC19A3 (−1,957 to +59 bp) promoters. In these investigations, SLC19A2 and SLC19A3 promoters fused to the Firefly luciferase reporter gene were transiently transfected into HuTu-80 cell lines and chronically exposed to ethanol (200 mM) (26) for 72 h. Luciferase activity was then measured as described by us previously (24, 28, 29). The results showed a significant ($P < 0.05$) reduction in the activity of the both SLC19A2 (Fig. 8A) and SLC19A3 (Fig. 8B) promoters in cells chronically exposed to alcohol compared with control cells.

**DISCUSSION**

This study was undertaken to determine the physiological and molecular mechanisms by chronic alcohol consumption leading to inhibition in intestinal thiamin absorption. It has been known for some time that chronic alcoholism is associated with thiamin deficiency, which may lead to Wernicke’s encephalopathy and Korsakoff’s psychosis (30, 41, 45). Although a number of factors may be involved in the pathogenesis of thiamin deficiency in the setting of chronic alcohol intake (e.g., inadequate intake of thiamin, decreased liver storage of the vitamin, urinary losses) (1, 17, 30), an inhibition in intestinal absorption of this vitamin plays an important role in this process given the central role of intestine in regulating normal body homeostasis of thiamin. We used two established models (in vivo and in vitro) of alcohol exposure to achieve our aims: a rat model in which alcohol was provided in a nutritionally adequate liquid diet and involved pair-feeding of controls and human-derived intestinal epithelial HuTu-80 cells chronically exposed to a clinically relevant concentration of alcohol.

Our studies with rats revealed that chronic alcohol consumption causes a significant inhibition in both the entry step of thiamin into the polarized enterocytes across the apical BBM and the exit step of the vitamin across the BLM. These findings confirm the previous suggestion that chronic alcohol feeding affects the carrier-mediated process of the intestinal thiamin uptake (17). The effect of alcohol on thiamin transport across the apical BBM was evident as early as 2 wk following the initiation of alcohol feeding. Furthermore, the inhibition of thiamin uptake across the BBM and BLM was associated with a decrease in the level of expression of the THTR-1 protein at both the BBM and BLM; expression of THTR-2 at the apical BBM was not affected. Of note, THTR-2 is only expressed at the BBM and not the BLM, as shown by us previously using confocal imaging and immunological approaches (4, 33) and confirmed in the present study (Fig. 4B). The decrease in protein abundance of THTR-1 protein in alcohol-fed rats was associated with a significant decrease in message level of the transporter. In contrast, THTR-2 protein and mRNA levels were not different in alcohol-fed animals compared with pair-fed controls. These findings demonstrate differential effects of chronic alcohol consumption on expression of thiamin transporters in the intestine, a finding that is in line with previous observations showing differential effects of chronic alcohol feeding on nutrient transport in the gut (11–13) and on gene expression (including those that encode membrane transporters) in other tissues (20). Furthermore, the observation that chronic alcohol feeding affects the expression of THTR-1 but not THTR-2 in the intestine contrasts our recent findings in the kidneys of rats chronically fed alcohol, where decreased levels of expression of both THTR-1 and THTR-2 were observed (40). These findings demonstrate that chronic alcohol feeding exerts a tissue-specific effect on the molecular parameters of thiamin transport. It is interesting to note here that chronic alcohol feeding also affects thiamin metabolism in a tissue-specific manner (31).

The decrease in the level of expression of THTR-1 mRNA in the intestine of rats chronically fed alcohol compared with their pair-fed controls suggests that the inhibition may (at least in part) be transcriptionally mediated. This possibility was tested by determining the level of expression of THTR-1 hnRNA in the jejunum of alcohol-fed rats compared with their pair-fed controls because changes in the level of hnRNA expression could serve as an indicator for changes in the rate of transcription of this gene (2, 8, 19).
The results showed that chronic alcohol feeding leads to a significant reduction in the level of THTR-1 hnRNA, thus supporting the possibility that the inhibitory effect of chronic alcohol feeding on intestinal thiamin uptake may (at least in part) be exerted at the transcriptional level. Obviously, this does not exclude the involvement of other mechanisms (e.g., changes in RNA stability), and further studies are needed to address this issue.

As mentioned earlier, the intestinal tract is exposed to two sources of thiamin: a dietary source that is absorbed in the small intestine and a bacterial source where the vitamin is generated by the normal microflora in the large intestine and is absorbed in that region of the gut via an efficient carrier-mediated mechanism (34). Thus in this study we also examined the effect of chronic alcohol feeding to rats on thiamin uptake in the colon and observed a significant inhibition in the uptake process. Thus chronic alcohol use appears to inhibit the absorption of both dietary and bacterial sources of thiamin. Because alcohol is absorbed mainly in the upper small intestine (26), it is reasonable to assume that the colon gets exposed to ethanol from the blood side (37). Also, because colonic bacteria can metabolize some of the ethanol that enters the colon to acetaldehyde (37) and because the colon has a low capacity to metabolize acetaldehyde secondary to a low level of aldehyde dehydrogenase activity (37), it is possible that some of the inhibitory effect of chronic alcohol feeding on colonic thiamin uptake is caused by this alcohol metabolite. Thus chronic alcohol feeding appears to inhibit the absorption of both dietary and bacterial sources of thiamin. The clinical implication of these findings would be that chronic alcohol consumption has a significant deleterious effect on the overall physiology of intestinal thiamin transport.

In our in vitro model, i.e., studies with the human-derived intestinal epithelial HuTu-80 cells chronically exposed to alcohol (26), also showed a significant inhibition in carrier-mediated thiamin uptake. This inhibition, however, was associated with a significant reduction in level of expression of both hTHTR-1 and hTHTR-2 at the protein and mRNA levels. This is unlike what we observed above in rats chronically fed alcohol where only THTR-1 appears to be affected. Whether this is due to differences in the model used (in vivo alcohol feeding vs. in vitro alcohol exposure) and/or due to interspecies difference is not clear and requires further investigations. The reduction in the level of expression of hTHTR-1 and hTHTR-2 appears to again be mediated (at least in part) via transcriptional mechanism(s), as indicated by the significant inhibition in the activity of the SLC19A2 and SLC19A3 promoters in human intestinal epithelial HuTu-80 cells chronically exposed to alcohol compared with control cells. Again, other mechanisms may contribute to the inhibitory effect of chronic alcohol exposure on thiamin uptake by HuTu-80 cells, and further investigations are needed to address this issue.

In summary, chronic alcohol feeding/exposure inhibits the physiological and molecular parameters of intestinal thiamin uptake process, and that effect is mediated (at least in part) via transcriptional mechanism(s). In addition, the inhibition in thiamin transport appears to be at the level of both the small and large intestine.

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DISCLOSURES
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