HHS Public Access

Author manuscript

Crit Care Med. Author manuscript; available in PMC 2016 September 01.

Published in final edited form as:

Crit Care Med. 2016 September ; 44(9): e875–e881. doi:10.1097/CCM.0000000000001745.

Inhibition of Intestinal Thiamin Transport in Rat Model of Sepsis

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Abstract

Objective—Thiamin deficiency is highly prevalent in patients with sepsis, but the mechanism by which sepsis induces thiamin deficiency is unknown. This study aimed to determine the influence of various severity of sepsis on carrier-mediated intestinal thiamin uptake, level of expressions of thiamin transporters (thiamin transporter-1 (THTR-1) and thiamin transporter-2 (THTR-2)), and mitochondrial thiamin pyrophosphate transporter (MTPPT).

Design—Randomized, controlled study

Setting—Research laboratory at a Veterans Affairs Medical Center

Subjects—Twenty-four Sprague-Dawley rats were randomized into controls, mild, moderate and severe sepsis with equal number of animals in each group.

Measurements and Main Results—Sepsis was induced by cecal ligation and puncture with the cecum ligated below the cecal valve at 25 %, 50 % and 75 % of cecal length, defined as severe, moderate and mild sepsis, respectively. Control animals underwent laparotomy only. After 2 days of induced sepsis, carrier-mediated intestinal thiamin uptake was measured using [^{3}H]thiamin. Expressions of THTR-1, THTR-2, and MTPPT proteins and mRNA were measured. Proinflammatory cytokines (IL-1β and IL-6), and adenosine triphosphate (ATP) were also measured. Sepsis inhibited [^{3}H]thiamin uptake and the inhibition was a function of sepsis severity. Both cell membranes thiamin transporters and MTPPT expression levels were suppressed; also levels of ATP in the intestine of animals with moderate and severe sepsis were significantly lower than that of sham operated controls.

Conclusions—For the first time we demonstrated that sepsis inhibited carrier-mediated intestinal thiamin uptake as a function of sepsis severity, suppressed thiamin transporters and MTPPT, leading to ATP depletion.

Keywords

Thiamin deficiency; Thiamin pyrophosphate; Intestinal Thiamin uptake; Thiamin transporter; sepsis; mitochondria

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INTRODUCTION

The active form of thiamin, thiamin pyrophosphate represents ~ 85–90% of total cellular thiamin, is generated exclusively in the cytoplasm, and transported into the mitochondria for utilization of cellular metabolic process. Thiamin pyrophosphate is indispensable for normal mitochondrial function and structural integrity due to its involvement as a co-factor in oxidative energy metabolism, adenosine triphosphate (ATP) production and maintenance of cellular redox state by reducing cellular oxidative stress (1). Thus, low intracellular levels of thiamin leads to impairment in oxidative energy metabolism (acute energy failure), a propensity for oxidative stress, and eventually apoptosis.

In critically ill adult and pediatric patients, thiamin deficiency is highly prevalent, ranged from 20 % to 70 % (2–4). In one retrospective study (5), the mortality rate of critically ill patients with thiamin deficiency was 72 % compared with 44 % without the deficiency. Thiamin deficiency appears to contribute to the high mortality, yet the risk factor(s) contributing to the deficiency were not investigated. In critically ill patients with sepsis, sepsis has been identified as an important risk factor for thiamin deficiency (2–4). However, the extent by which sepsis leads to thiamin deficiency is unknown. Thiamin is not synthesized endogenously, but has to be obtained exogenously from nutrient via transport across the intestinal cell membrane, or from the circulation via transport to other organs. The cell membrane thiamin transporters (THTR), THTR-1 (encoded in *SLC19A2* gene) and THTR-2 (encoded in *SLC19A3* gene) mediate intestinal thiamin transport process, whereas the uptake of thiamin pyrophosphate into the mitochondria occurs via a carrier-mediated mechanism that involves the mitochondrial thiamin pyrophosphate transporter (MTPPT, encoded in *SLC25A19* gene) (6). The effect of sepsis on intestinal thiamin uptake, and its influence on intestinal thiamin transporters, THTR-1 and THTR-2, and MTPPT is unknown. We hypothesize that sepsis produces thiamin deficiency, at least in part, by inhibition of thiamin uptake via suppression of intestinal thiamin transporters. Furthermore, sepsis suppresses MTPPT resulting in reduced ATP generation. The objective of this study is to investigate in experimental model of sepsis in rats, the influence of sepsis and degree of severity on intestinal thiamin uptake, and the state of thiamin transporters in cytoplasm and mitochondria.

MATERIAL AND METHODS

Subjects, surgical procedures, animal monitoring and care

This study received approval from the Research and Development Subcommittee on Animal Studies of the Veterans Affairs Long Beach Healthcare System. We studied a total of 24 male pathogen-free Sprague-Dawley rats randomized into groups of sham control, severe, moderate and mild degree of sepsis with equal number of animals in each group. Sepsis was produced using the cecal ligation and puncture (CLP) technique (7). The severity of sepsis was based on the extent of cecal ligation (see below) (7). Surgeries were performed under general anesthesia with an intramuscular mixture of ketamine hydrochloride 100 mg/kg and xylazine 5 mg/kg. A 2-cm midline incision of the skin and peritoneum was made in the lower abdomen. The cecum was identified, withdrawn through the incision, and was ligated below the cecal valve at 25%, 50% and 75% of the cecal length with a 3–0 silk suture to

define severe, moderate and mild degree of sepsis, respectively. Using an 18-gauge needle, the cecum was perforated in two locations on the anti-mesenteric surface and was gently compressed until feces were extruded to ensure patency of the holes. The sham control animals underwent similar procedures without the cecal ligation and puncture. During surgery, rectal temperature was monitored, and maintained at 37°C using homeothermic warming pad (Right-Temp, Kent Scientific Corp, Torrington, CT) and a heating lamp. Blood pressure was monitored noninvasively using the tail cuff method (CODA Monitor, Kent Scientific Corp). Respiration, heart rate, and arterial oxygen saturation (SpO2) were monitored and displayed in real time using pulse oximeter (MouseSTAT, Kent Scientific Corp). The animals were allowed to recover for 2 d. Post-operatively, rectal temperature, blood pressure, respiration, and heart rate were measured twice a day. Antibiotic (Gentamicin 0.5 mg/kg I.M. twice a day), and analgesic (Buprenorphine 0.05 mg/kg S.C. every 6 h) were administered immediately following surgery and for 2 d. Pre-warmed 0.9% NaCl at 50 mL/kg S.C. was administered twice a day and as needed to maintain mean blood pressure of 70 mm Hg. Liquid acetaminophen 0.1 mg/mL was mixed in the drinking water. Softened rat chow was provided liberally, however when oral intake was poor, animals were hand-fed twice a day with Ensure Complete 10 ml/kg (Abbott Nutrition, Columbus, OH). Animals that died, mostly due to hypotension, were replaced to maintain equal number of animals in each group (n = 6).

Measurement of thiamin uptake

After 2 days of sepsis, under general anesthesia, animals underwent laparotomy, and thiamin uptake measurement in the proximal jejunum using [^{3}H]thiamin. In vivo, two intact jejunal loops (2 cm each in length) separated with another jejunal loop of the same length were prepared by suture ligation. In the first segment, 250 μL of Krebs–Ringer buffer containing [^{3}H]thiamin was injected intraluminally, and in the second segment, labeled and unlabeled thiamin mixed with the same incubation medium were injected.

Uptake of [^{3}H]thiamin (250 μM) by the carrier-mediated process was determined by subtracting uptake in the presence of a high pharmacological concentration of unlabeled thiamin (1000 μM) from uptake in its absence. Radioactivity uptake counting was measured after 5 minutes with Beckman Counter LS 6500 (linear phase of uptake). Uptake data were expressed in fmol/mg tissue wet weight/5 min. All thiamin uptake experiments in septic rats were run simultaneously with weight-matched control rats. Following thiamin uptake measurements, blood sample was withdrawn from inferior vena cava for thiamin and cytokines determination. Rats were then euthanatized with pentobarbital 200 mg/kg, i.p.

Determination of thiamin blood concentration and level of serum cytokines

Whole blood thiamin concentration was measured using a microbiological test kit with Lactobacillus fermentum coated microtiter plate (Institut für Produktqualität GmbH, ID-Vit Vitamin B1 KIF001, Berlin, Germany). Mean reference value of whole blood thiamin was 48.1 ± 8.9 μg/L (± SD). Serum cytokines (IL-1β and IL-6) were measured using ELISA kit (Thermo Fisher Scientific, Grand Island, NY).

Determination of Adenosine triphosphate (ATP)

ATP in the jejunal tissue was measured colorimetrically using ATP assay Kit (ab83355; Abcam, Cambridge, MA) (8). Following dissection, tissue was flash frozen by immediate immersion in 100% isopentane (Mallinckrodt Baker Inc., Phillipsburg, NJ) pre-chilled on dry ice. After tissue homogenization, samples were then deproteinated and neutralized by adding ice-cold Neutralization Solution (ab93299; Abcam) at 1:24 (v:v), incubating on ice for 5 min, centrifuging at 10,000g for 2 min and mixing with ice-cold ATP Assay Buffer at 1:5 (v:v). ATP standard curve was created, and ATP concentration in the tissue lysed from either controls or septic animals was calculated relative to the standard curve, and normalized for protein concentration.

Determination of THTR-1, THTR-2 and MTPPT protein and mRNA

Frozen intestine tissues were processed for determination of THTR-1, THTR-2, and MTPPT protein abundance using standard western blotting. The intestine (40 mg) from the septic rats and paired controls were homogenized in protein buffer (T-PER78510, Thermo Scientific, Rockford, lL) and denatured for THTR-1 and THTR-2 protein analysis. Mitochondrial protein was extracted for MTPPT protein measurement. We employed Ponceau S as reference protein (9). The immunoreactive bands were analyzed using ImageQuant software, version 5.0 (Molecular Dynamics, Sunnyvale, CA).

For mRNA extraction, approximately 30 mg frozen intestine tissue was homogenized in TRizol Reagent (Invitrogen, Carlsbad, CA, USA) and total RNA was isolated according to the manufacturer's protocol. Following reverse transcription, the mRNA expression level was quantified in a real-time PCR system (CFX384 Touch™ Bio-Rad Laboratories, Hercules, CA) using SYBR Green Super mix (Bio-Rad Laboratories), and was performed in triplicate. 18S was used as the housekeeping gene. Specific primers for rats are as follows: THTR-1: forward 5'-GATGCTCCTACGTACTGCCC-3' and reverse 5'-GCAGGTAGGGAGTGAGGAAC-3'; THTR-2: forward 5'-TGATACTCTGCTTGTCGG-3' and reverse 5'-GTAAGAGTACGTCCAAACAG-3'; MTPPT: forward 5'-GGCCATACGCACCATG-3' and reverse 5'-GGGTCTTGCTGATGACTC-3', and 18S: forward 5'-GCAGAATTCCCACTCCCGACCC-3' and reverse 5'-CCCAAGCTCCAACTACGAGC-3'. Data were normalized to 18S and calculated using a relative relationship method supplied by the manufacturer (Bio-Rad Laboratories).

Statistical Analysis

Group size was determined from thiamin uptake values from the initial 3 animals based on minimum differences in a mean of 0.30 and expected standard deviation of residuals of 0.15. We required 6 animals per group to achieve a power of 0.80 and a $\alpha = 0.05$ (ANOVA; SigmaPlot, version 12.0; SPSS, Inc., Chicago, IL). Values were expressed as mean ± SE unless specifically indicated. A two-way ANOVA was used to compare physiological variables among groups using the appropriate grouping variables of time (baseline and day 2), and severity of sepsis (mild, moderate and severe as defined above). For other variables, one-way ANOVA was used for comparison among groups. When the F value was significant, post-hoc analysis was performed using the Tukey test for pairwise multiple comparisons. Group differences were considered significant at $P \leq 0.05$.

RESULTS

The sepsis model employed in the present study resulted in animal survival rate of 100 %, 90 %, 86 % and 75 % in sham control, mild, moderate and severe sepsis groups, respectively. At baseline, compared with controls, body weight (BW), mean blood pressure, heart rate, respiratory rate, and SpO2 were similar among groups, except body temperature was higher in all of the sepsis groups (Table S1 in supplemental digital content (SDC)). After 2 days, BW tended to decrease in moderate and severe sepsis. Body temperature returned to levels that of controls, likely related to antibiotic administration. Hemodynamic, respiratory and oxygenation variables were stable. The degree of pro-inflammatory cytokines (IL-1β and IL-6) production is a function of sepsis severity (Table 1). Thiamin blood concentration in the septic animals was not significantly different from controls (Table 1).

Carrier-mediated thiamin uptake was reduced significantly in proportion to the severity of sepsis (Figure 1). This was further supported by the significant correlation coefficient between thiamin uptake and IL-1β ($r^2 = 0.58$, $P < 0.001$), and IL-6 ($r^2 = 0.67$, $P < 0.001$) (Figure S1 in SDC). As thiamin pyrophosphate is essential for the conversion of pyruvate to acetyl-CoA in the generation of ATP, the reduced intestinal thiamin uptake led to a significantly decreased ATP in the moderate and severe sepsis groups, by 52 % and 58 %, respectively (Table 1).

The uptake of thiamin in the intestine is an active process mediated via cell membrane THTR-1 and THTR-2. As demonstrated in Figure 2, sepsis decreased both THTR-1 and THTR-2 protein significantly. Similarly, sepsis suppressed both THTR-1 and THTR-2 mRNA expressions (Figure 3). The active form of thiamin, thiamin pyrophosphate, also requires a carrier-mediated transport for entry into the mitochondria. As shown in Figure 4, sepsis significantly reduced MTPPT protein and suppressed its mRNA expressions.

DISCUSSION

Sepsis is a systemic inflammatory response to infection with a prevalence for severe sepsis of 25 to 44 %, and a mortality rate of approximately 30% or greater (10). Few observational studies in critically ill patients have identified sepsis (2–4) as an important risk factor contributing to thiamin deficiency, and that the combined sepsis and thiamin deficiency was associated with higher mortality than sepsis alone (3). The present study is the first to demonstrate that 1) sepsis inhibited intestinal carrier-mediated thiamin uptake, and that the inhibition was in proportion to the severity of sepsis (Figures 1); 2) both thiamin transporters protein and mRNA expression levels were suppressed (Figures 2 and 3, respectively); and most importantly, 3) MTPPT protein and mRNA expressions were markedly decreased and associated with proportionate reduction in ATP in animals with moderate and severe sepsis.

Our animal model of sepsis mimicked human sepsis. The mortality rate of animals with severe sepsis was 25%, close to that reported mortality in human with sepsis (10). Contrasts to a similar rat model of severe sepsis, the present study had a significantly lower mortality rate than that previously reported after 2 days of induced sepsis, 25 % versus 70 %,

respectively (7). The reduced mortality rate in our study was likely related to the use of systemic antibiotic administered immediately following surgery and maintained for 2 days.

Gut-derived sepsis, the sepsis model employed, has been shown to impair intestinal epithelial barrier function to promote bacterial translocation into the systemic circulation with subsequent bacteremia, and pathologic inflammation of the intestinal mucosa (11). Whether inflammation of the intestinal mucosa alone with or without adherence of the organisms to the intestinal epithelium is a requirement for thiamin uptake inhibition is unclear. Previous in vitro studies from our laboratory demonstrated that exposure of human-derived intestinal epithelial cells to enteropathogenic *Escherichia coli* (12) or toxin-producing *Escherichia coli* (13) resulted in significant inhibition of thiamin uptake. Notwithstanding the mechanisms of intestinal thiamin uptake inhibition, the present study demonstrated that sepsis is an important risk factor for the development of thiamin deficiency as previously reported in critically ill patients (2, 4). Sepsis not only inhibited intestinal thiamin uptake and transporters, but the inhibition was a function of sepsis severity. Keeping with previous report in severe septic patients (4), the prevalence of thiamin deficiency was very high at 70 %.

Thiamin uptake inhibition in sepsis was also associated with THTR-1 and THTR-2, as well as MTPPT suppression at the protein and mRNA levels (Figures 2–4). With respect to mRNA suppression, it is unclear at present whether the reduction in mRNA of these transporters is mediated at the transcriptional and/or post-transcriptional levels (e.g., changes in mRNA stability). Further studies are needed to address these issues.

Compared with controls, the significant inhibition of carrier-mediated thiamin uptake in the septic animals was not associated with reduced blood thiamin concentration or thiamin deficiency. This observation was not surprising as our animal model of sepsis was maintained for only 2 days, shorter than thiamin biologic half-life of 9 to 18 days (14). Additionally, unlike critically ill patients with sepsis whose premorbid conditions may be associated with thiamin deficiency (e.g., chronic alcoholism (15), diabetes (16), celiac disease (17), or long-term use of furosemide diuretic (18)), our animals were healthy prior to the induction of sepsis. Our observation suggests that a normal thiamin blood level in sepsis does not preclude functional thiamin deficiency state, defined as inhibition of carrier-mediated thiamin uptake associated with suppression of thiamin transporters in the cell membrane and mitochondria. Yet, in pre-existing thiamin deficiency, the effect of sepsis on carrier-mediated thiamin uptake and its transporters is unknown and requires further study. This is because adaptive regulation to thiamin deficiency occurs. In mice fed with thiamin deficient diet, carrier-mediated thiamin uptake was upregulated as a result of increased expressions of THTR-2 at both the protein and mRNA levels, without changes in THTR-1 expressions (19). Depending on sepsis severity, it is possible that in pre-existing thiamin deficiency, carrier-mediated thiamin uptake and THTR-2 expression may be preserved. However, the septic-induced suppression of THTR-1 as shown in the present study, may offset the adaptive regulation of THTR-2.

Sepsis did not only depressed THTR-1 and THTR-2, but also reduced the expression of MTPPT considerably in both moderate and severe sepsis groups (Figure 4). In the

mitochondria, thiamin pyrophosphate is a co-factor for pyruvate dehydrogenase in the conversion of pyruvate to acetyl-CoA, and α-ketoglutarate dehydrogenase in the conversion of α-ketoglutarate to succynil-CoA in the tricarboxylic acid cycle (Krebs cycle) for the generation of ATP. Our study showed a significantly reduced ATP generation in animals with moderate and severe sepsis. While sepsis itself, via oxidative stress (11) inhibits electron transport chain complexes activity (20), another pathway for ATP generation independent of thiamin; in the present study, the reduced MTPPT protein or mRNA expressions contributed to the reduced ATP production. Levels of MTPPT protein and mRNA expressions were responsible for 50% of the variance in ATP production as shown in the significant correlation between ATP production and MTPPT protein and mRNA expression levels ($r^2 = 0.50$; $P < 0.01$ for MTPPT protein; and $r^2 = 0.54$; $P < 0.01$ for MTTPT mRNA expression, respectively).

ATP is essential to provide energy for numerous cellular processes and reactions (21), particularly in the metabolically active cells such as the intestine for nutrient absorption. As sepsis is a systemic inflammatory syndrome, the reduced ATP generation may not be limited to the intestinal epithelial cells, but may involve other organs via similar mechanism of impaired transport of thiamin pyrophosphate into the mitochondria. Further research is needed to investigate the role of sepsis on functional thiamin deficiency with its consequence of ATP depletion in other organs such as the brain in the manifestation of sepsis-induced delirium (22), or in the peripheral nerves and skeletal muscles in sepsis-induced ICU acquired muscle weakness.

Limitation of the study

We defined severity of sepsis according to the site of cecal ligation and not according to the extent of organ failure or tissue hypoperfusion as clinically defined (23). However, animals with severe sepsis had the highest mortality and associated with the highest proinflammatory cytokines levels (Table 1) supporting the severity of sepsis. The duration of sepsis was limited to 2 days. A temporal relationship between duration of sepsis and progression of thiamin deficiency is unknown, and requires further study.

Our measurement of thiamin was obtained from whole blood using microbiological test method rather than using high performance liquid chromatography as used clinically (24), however, performance of these two methods are comparable (25).

Clinical implication

Alcoholism has been implicated as the most common predisposing factor for thiamin deficiency with its complications of Wernicke-Korsakoff syndrome and peripheral neuropathy (26). As reported in previous clinical studies (2–5), the results of our study confirmed that sepsis, particularly severe sepsis, is an important risk factor for the development of functional thiamin deficiency via, at least in part, inhibition of carrier mediated intestinal thiamin uptake. Normal thiamin blood concentration does not preclude functional thiamin deficiency in specific organ. In sepsis, reduced thiamin blood concentration suggests a late manifestation of thiamin deficiency state and may portend a poor prognosis. Further prospective study is needed to confirm this contention.

Conclusion

Sepsis induced inhibition of intestinal thiamin uptake; the latter was a function of sepsis severity. Sepsis also suppressed thiamin transporters (THTR-1 and THTR-2), and MTPPT, leading to a significant reduction in ATP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by the Department of Veterans Affairs Medical Research Service awarded to C.S. Sassoon and H.M. Said, and National Institutes of Health (DK-56061, DK-58057, AA-018071) awarded to H.M. Said. The work was conducted in the Department of Research and Development, VA Long Beach Healthcare System, Long Beach, CA.

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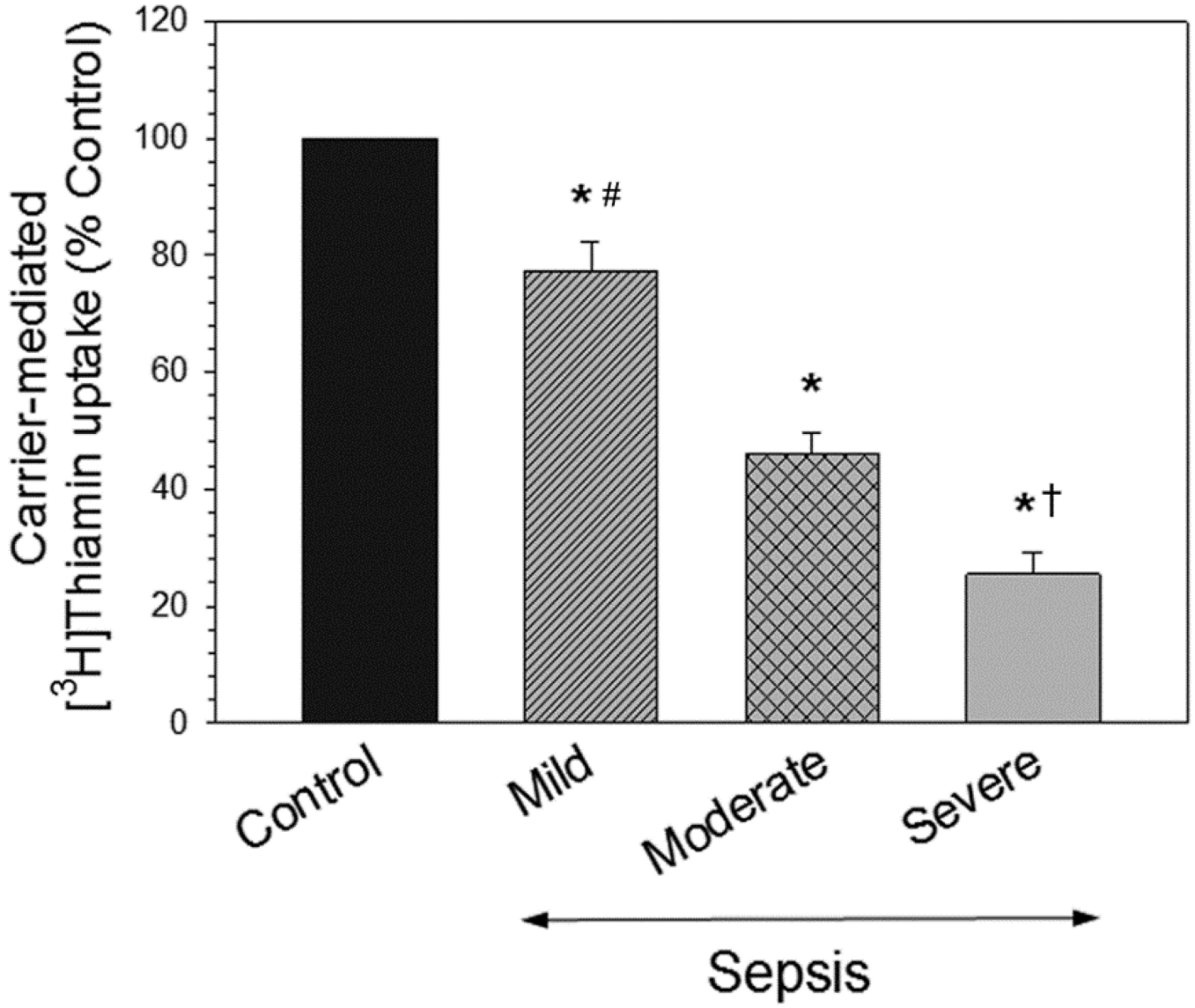

FIGURE 1.

Intestinal carrier-mediated thiamin uptake. Thiamin uptake expressed as percent of controls. Values are mean ± SE; n = 6 for each group.

*P < 0.001: severe compared with mild sepsis and control; moderate compared with mild sepsis, and control.

†P = 0.003: severe compared with moderate sepsis.

#P = 0.001: mild sepsis compared with control.

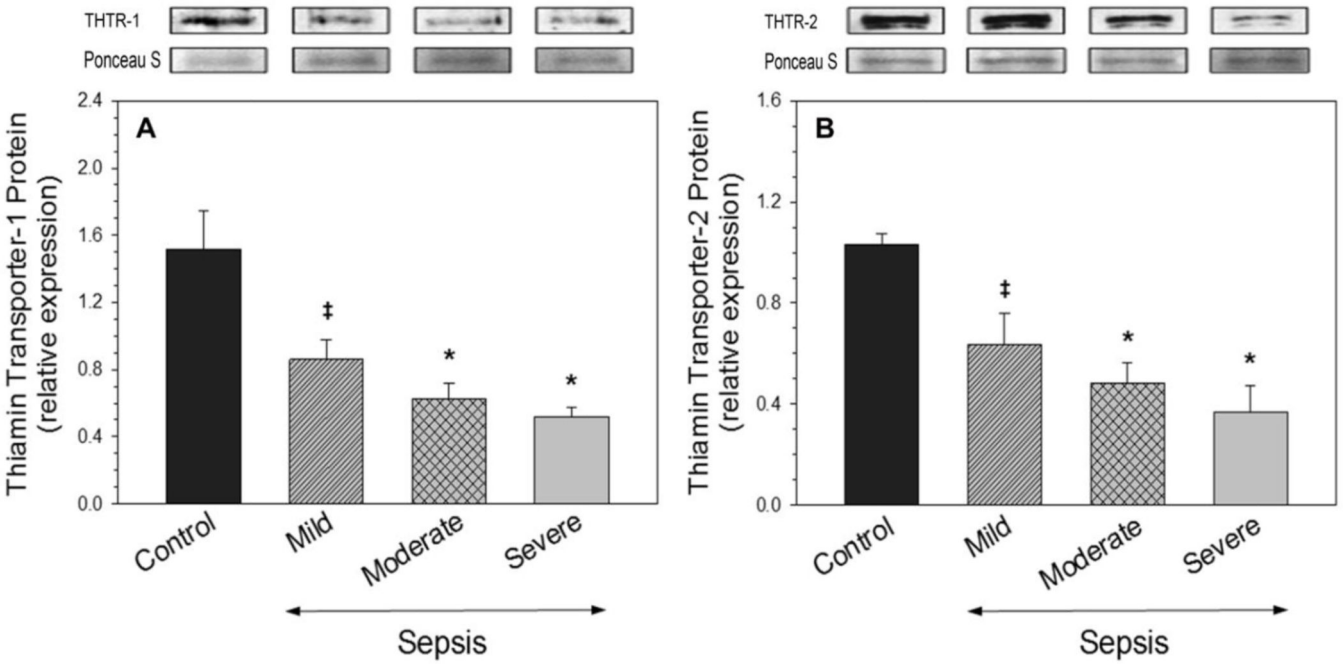

FIGURE 2.

Thiamin transporter-1 (panel A) and thiamin transporter-2 (panel B) proteins.

Values are mean ± SE; n = 4 for each group. Representative gel images of respective proteins of different groups were obtained from the same gel.

Thiamin tranporter-1:

* P = 0.002 severe compared with control.

* P = 0.004 moderate sepsis compared with control.

‡ P = 0.027 mild sepsis compared with control.

Thiamin transporter-2:

* P = 0.002 severe sepsis compared with control.

* P = 0.006 moderate sepsis compared with control.

‡ P = 0.047 mild sepsis compared with control.

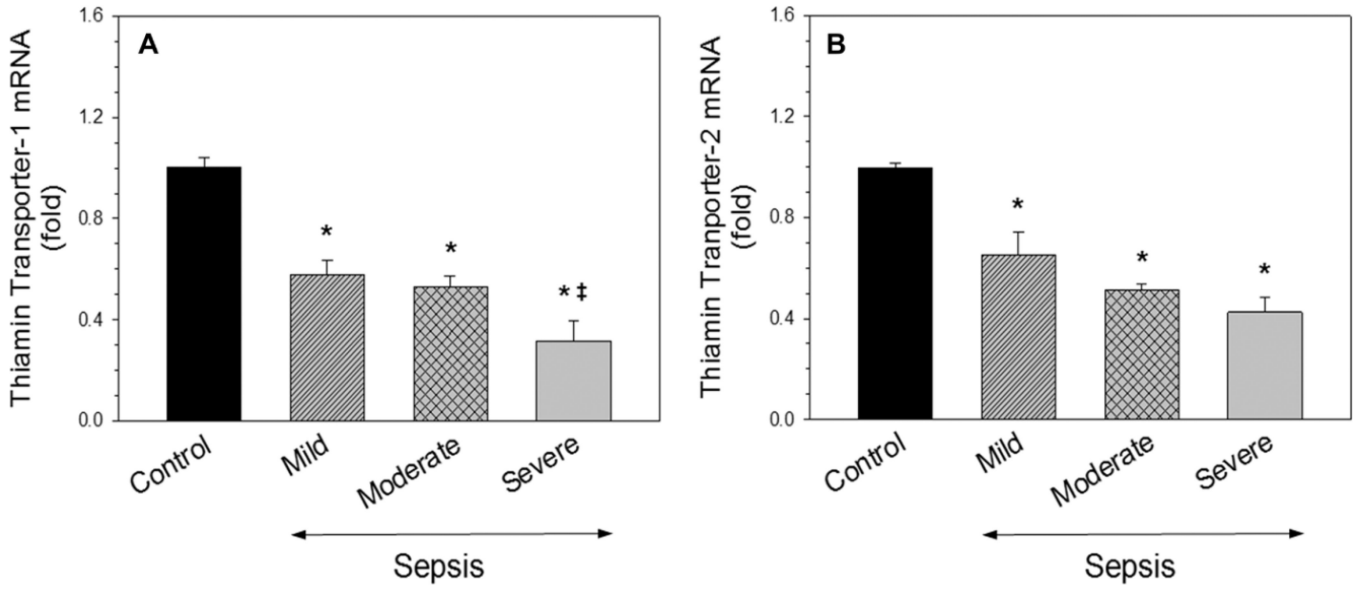

FIGURE 3.

Thiamin transporter-1 (panel A) and thiamin transporter-2 (panel B) mRNA.

Values are mean ± SE; n = 4 for each group.

Thiamin tranporter-1:

* P < 0.001 severe and moderate sepsis compared with control;

* P = 0.001 mild sepsis compared with control.

‡ P = 0.026 severe compared with mild sepsis.

Thiamin transporter-2:

* P < 0.01 severe and moderate sepsis compared with control.

* P = 0.005 mild sepsis compared with control.

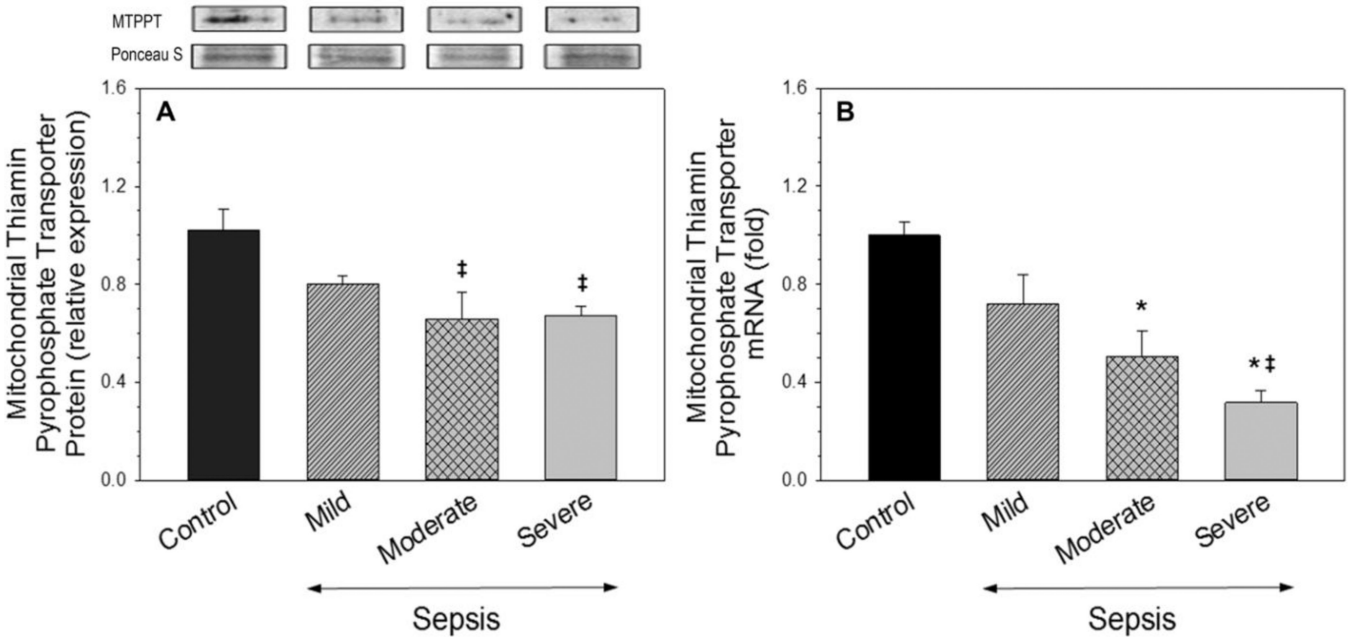

FIGURE 4.

Mitochondrial thiamin pyrophosphate transporter protein (panel A) and mRNA (panel B). Values are mean ± SE; n = 4 for each group. Representative gel images of MTPPT proteins of different groups were obtained from the same gel.

Mitochondrial thiamin pyrophosphate transporter protein:

‡ P = 0.028 severe sepsis compared with control.

‡ P = 0.022 moderate sepsis compared with control.

Mitochondrial pyrophosphate transporter mRNA:

* P < 0.001 severe sepsis compared with control.

* P = 0.004 moderate sepsis compared with control.

‡ P = 0.021 severe compared with mild sepsis.

Table 1

Serum cytokines, whole blood thiamin concentration and intestine adenine-triphosphate in control and septic animals

	Control	Sepsis		
		Mild	Moderate	Severe
IL-1β (pg/mL)	5.5 ± 1.4	38.4 ± 2.9	47.2 ± 6.6	148.9 ± 24.7 *
IL-6 (pg/mL)	11.8 ± 4.6	78.4 ± 15.8	143.8 ± 12.8 †	465.1 ± 46.2 *
Thiamin (μg/L) §	51.9 ± 1.1	52.5 ± 0.8	51.1 ± 1.3	50.6 ± 0.3
ATP (nmol/mg protein)	12.9 ± 1.4	10.9 ± 1.1	6.2 ± 0.4 *‡	5.4 ± 0.4 *

Values are mean ± SE; n = 6,

§ thiamin blood concentration n = 4.

IL-1β: *P < 0.001 severe sepsis compared with control, mild and moderate sepsis.

IL-6: *P < 0.001 severe sepsis compared with control, mild and moderate sepsis;

† P < 0.008 moderate sepsis compared with control.

Adenosine tri-phosphate (ATP):

* P < 0.001 severe and moderate sepsis compared with control.

* P = 0.003 severe compared with mild sepsis.

‡ P = 0.01 moderate compared with mild sepsis.