Original Communication

HIF1-α-Mediated Gene Expression Induced by Vitamin B_1 Deficiency

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Received: June 7, 2013; Accepted: October 16, 2013

Abstract: It is well established that thiamine deficiency results in an excess of metabolic intermediates such as lactate and pyruvate, which is likely due to insufficient levels of cofactor for the function of thiamine-dependent enzymes. When in excess, both pyruvate and lactate can increase the stabilization of the hypoxia-inducible factor 1-alpha (HIF-1α) transcription factor, resulting in the trans-activation of HIF-1α regulated genes independent of low oxygen, termed pseudo-hypoxia. Therefore, the resulting dysfunction in cellular metabolism and accumulation of pyruvate and lactate during thiamine deficiency may facilitate a pseudo-hypoxic state. In order to investigate the possibility of a transcriptional relationship between hypoxia and thiamine deficiency, we measured alterations in metabolic intermediates, HIF-1α stabilization, and gene expression. We found an increase in intracellular pyruvate and extracellular lactate levels after thiamine deficiency exposure to the neuroblastoma cell line SK-N-BE. Similar to cells exposed to hypoxia, there was a corresponding increase in HIF-1α stabilization and activation of target gene expression during thiamine deficiency, including glucose transporter-1 (GLUT1), vascular endothelial growth factor (VEGF), and aldolase A. Both hypoxia and thiamine deficiency exposure resulted in an increase in the expression of the thiamine transporter SLC19A3. These results indicate thiamine deficiency induces HIF-1α-mediated gene expression similar to that observed in hypoxic stress, and may provide evidence for a central transcriptional response associated with the clinical manifestations of thiamine deficiency.

Key words: thiamine deficiency, HIF-1α, pseudo-hypoxia

Introduction

Vitamin B1 (thiamine), and in particular thiamine pyrophosphate (TPP), is an essential cofactor required to maintain cellular metabolism [1]. The inability of mammals to synthesize thiamine *de novo* necessitates a continuous dietary intake to satisfy cellular metabolic requirements. Thiamine deficiency (TD) typically arises from malnutrition, consuming a diet high in anti-thiamine factors such as thiaminase, and in chronic diseases such as alcoholism, cancer, HIV, and gastrointestinal diseases [2]. The most well known disorders associated with thiamine deficiency include beriberi and Wernicke-Korsakoff syndrome [3]. Regardless of etiology, the clinical manifestations of thiamine deficiency are commonly metabolic acido-

 DOI 10.1024/0300–9831/a000159

sis, encephalopathy, optic neuropathy, and peripheral neuropathy[4, 5].

There are a number of potential mechanisms for TD-induced encephalopathy, with most centralized on the consequences of reduced thiamine-dependent enzyme activity [6,7]. Biochemically, TD reduces the activities of pyruvate dehydrogenase (PDH) and alpha-ketoglutarate dehydrogenase (α-KGDH) [7–9]. This is consistent with the observed clinical manifestations in TD patients that demonstrate an increase in blood and urinary pyruvate levels and lactic acidosis [10,11]. The accumulation of lactate and resulting tissue acidosis has been suggested to be a major contributor to neuronal cell death in TD-related disorders [12]. Moreover, a build-up of intracellular pyruvate has been shown to be pro-apoptotic and may further contribute to neural degeneration [13].

The end products of glycolytic metabolism, pyruvate and lactate have been shown to have a significant influence on the cellular signaling that regulates cell metabolism. When in excess, both pyruvate and lactate can increase the stabilization of the hypoxia-inducible factor 1-alpha (HIF-1α) transcription factor, independent of low oxygen, a condition that is termed pseudohypoxia [14]. HIF-1α mediated pathways facilitate a Pasteur effect on cellular metabolism, increasing glycolysis and conversion of pyruvate to lactate [15]. Aside from effects on metabolism, hypoxic signaling pathways may also induce apoptosis and necrosis [16]. Comparable necrotic lesions were found in patients with hypoxia/ischemia and TD in the thalamus and mammillary bodies, suggesting congruency between the cellular response to hypoxic and TD stress [17, 18]. Therefore, the objective of this work was to determine whether thiamine deficiency stabilizes HIF-1α and induces expression of HIF-1α regulated genes.

Materials and Methods

Materials

All cell culture reagents including trypsin/EDTA, penicillin/streptomycin, and RPMI 1640 were obtained from Mediatech (Manassas, VA). Fetal bovine serum (FBS) was purchased from PAA Laboratories (Dartmouth, MA). Cell culture-treated flasks, plates, and dishes were from Greiner Bio-one (Monroe, NC). Thiamine hydrochloride and pyrithiamine hydrobromide were purchased from Sigma-Aldrich (St. Louis, MO). Thiamine-deficient RPMI 1640 was custom formulated by Mediatech (Manassas, VA).

Cell Culture

The SK-N-BE neuroblastoma cell line was used throughout this study as a model for the effects of thiamine deficiency on neuronal cells [19, 20]. The cells were obtained from ATCC (Manassas, VA) and maintained in TD medium supplemented with 30 nM thiamine hydrochloride, 10 % FBS, and 1 % penicillin/streptomycin and referred to as T30 medium.

Hypoxia Exposure

Hypoxic treatments were conducted at 37°C in an atmosphere of 1 % O_2 and 5 % CO_2 in an incubator outfitted with a ProOX oxygen controller (Biospherix, Lacona, NY) supplying nitrogen gas. Oxygen levels were monitored daily using a Bacharach Fyrite® Gas Analyzer (Bacharach, Inc., Pittsburgh, PA) and the ProOX oxygen sensor calibrated weekly. All T30 media used for hypoxia experiments were pre-equilibrated in the hypoxia incubator at 1 % O_2 for a minimum of 24 hours before use. SK-N-BE cells were seeded into 100-mm culture dishes and allowed to grow until approximately 60 % confluency in normoxic conditions, after which medium was removed and hypoxia-conditioned medium was added, with cells placed in the hypoxia incubator for various times.

Thiamine Deficiency

Pyrithiamine hydrobromide was supplemented into thiamine-deficient medium to aid in the induction of TD [21]. Cells were cultured with thiamine-deficient RPM1 1640 medium supplemented with 50 μM pyrithiamine hydrobromide, 10 % FBS, and 1 % penicillin/streptomycin, referred to as PTD medium. Cells were grown until approximately 60 % confluency in T30 medium at which time medium was removed and replaced with PTD medium for the indicated times.

Inhibition of HIF-1α-mediated gene expression

To attenuate HIF-1α-mediated responses, cells exposed to hypoxia and PTD were treated with the HIF-1α inhibitor YC1 and a dominant negative HIF-1α construct [22, 23]. Cells were pre-treated with 25 μM YC1 for 24 hours, after which medium was removed and appropriate treatment medium was added in addition

to 10 μM YC1. All treated samples were exposed to 10 μM YC1 for a total of 5 days. PTD treatments were started on the same day as the 10 μM YC1 and lasted 5 days, while 1 % O_2-treated samples were exposed to 10 μM YC1 3 days prior to starting the 48-hour 1 % O_2 treatment to ensure all cells were exposed to YC1 for the same time periods.

The dominant negative form of HIF-1α lacking a DNA-binding domain, transactivation domains, and an oxygen-dependent degradation domain of HIF-1α was cloned from human reference cDNA using previously reported primer sets [24]. Cloned fragments were recovered and ligated into pcDNA3.1 (Invitrogen, Grand Island, NY) (HIF-1α-DN). Sequence was verified using the University of Georgia Genomics facility. Cells were grown to ~60 % confluency, medium was replaced and the transfection complex of 2.5 μg plasmid and 5 μL Metefectene Pro (Biontex Laboratories, San Diego, CA) was added as per manufacturer's protocol. After 24-hour incubation, medium was removed and treatment media was added.

Pyruvate and Lactate Assay

Pyruvate (intracellular) and lactate (intracellular and extracellular) were quantified in SK-N-BE cells using a pyruvate assay kit (Eton Bioscience, San Diego, CA) and an L-lactate Assay Kit (Eton Bioscience, San Diego, CA) after PTD treatment. Pyruvate and lactate were quantified as per manufacturer's protocol using whole cell lysates (WCL). Isolation of WCL was achieved using 1 % NP-40, 0.5 % sodium deoxycholate, 0.1 % sodium dodecyl sulfate (SDS) in a pH=8.5 50 mM Tris, 250 mM NaCl, 1 mM EDTA buffer supplemented with protease inhibitor cocktail (EMD Biosciences, La Jolla, CA). Lysates were centrifuged at 16,000 x *g* for 20 minutes at 4°C and the supernatant was collected. Protein concentrations were determined using the BCA Protein Assay Kit (Thermo Scientific, Rockford, IL). Briefly, 50 μL of samples and standards were loaded into a 96-well plate followed by the addition of 50 μL of the supplied reaction reagent. The plate was then incubated at 37°C for 30 minutes and pyruvate was assayed using fluorescence measured at E_x=544 nm and E_m=590 nm and lactate was assayed by measuring the absorbance at λ = 490 nm using a Spectramax M2E plate reader (Molecular Devices, Sunnyvale, California). Results were normalized to total protein measured using the BCA Protein Assay (Thermo Scientific, Rockford, IL).

Real-time quantitative PCR (qRT-PCR)

RNA was isolated after either hypoxia or PTD treatments using the EZNA Total RNA Kit I (Omega Bio-tek, Norcross, GA) and reverse transcribed (1 μg) to cDNA using qScript cDNA Synthesis Kit as per manufacturers protocol (Quanta Biosciences Inc., Gaithersburg, MD). Primers for each gene were designed using the Roche Universal ProbeLibrary website to correspond with a specific 8–9 nucleotide hydrolysis probe labeled at the 5' end with fluorescein (FAM). Probes and primers used were #72 for SLC19A2 (F-GACACCCCAGCTTCTAACCA, R-AAGGAGACGGTCTGGCTTG), #5 for SLC19A3 (F-AATGGGGCCGTAGAAGCTAT, R-TTTCACATAACCCACTGCAAA), #81 for glucose transporter-1 (GLUT1) (F-GCCCATGTATGTGGGTGAA, R-AGTCCAGGCCGAACACCT, #12 for vascular endothelial growth factor (VEGF) (F-CAGACTCGCGTTGCAAGA, R-GAGAGATCTGGTTCCCGAAA), and #66 for Aldolase A (F-TGCCAGTATGTGACCGAGAA, R-GCCTTCCAGGTAGATGTGGT). The TATA-box-binding protein (TBP) reference assay kit supplied by Roche Applied Science was used as a housekeeping gene. The fold-change in gene expression comparing untreated and treated SK-N-BE cells was calculated using the comparative $2^{-\Delta\Delta Ct}$ method [25].

Western blotting

The protein expression of HIF-1α was assessed by Western blot in SK-N-BE cells after PTD and 1 % O_2 exposure. For isolation of nuclear lysates, cells were first lysed with 10 mM HEPES, 10 mM KCl, 0.1 mM EDTA, and 0.1 mM EGTA pH = 7.9 and protease inhibitor cocktail on ice for 15 minutes. Afterward, 10 % NP-40 was added at a ratio of 62.5 μL/mL of lysate then mixed and set on ice for an additional 5 minutes. Lysates were then centrifuged at 16,000 x *g* for 10 minutes at 4°C and the supernatant was discarded. The pellet was resuspended in 20 mM HEPES, 0.4 M NaCl, 1.0 mM EDTA, and 1.0 mM EGTA pH = 7.9 containing protease inhibitor cocktail and incubated on ice for 40 minutes, vortexing every 10 minutes for 30 seconds. The sample was then centrifuged at 16,000 x *g* for 5 minutes at 4°C and the resulting nuclear supernatant collected. Protein concentrations were determined using the BCA Protein Assay Kit (Thermo Scientific).

Nuclear lysates (50 μg) were separated on a 10 % SDS-PAGE gel and transferred to a polyvinylidene

difluoride membrane (GE Healthcare, Piscataway, NJ). The membrane was blocked with 5 % non-fat dry milk in Tris-buffered saline-Tween 20 (TBS-T) for 1 hour. The membrane was then immunoblotted for HIF-1α (rabbit anti-human, Bethyl Laboratories, Inc., Montgomery, TX) at 1:500 overnight. The blot was washed 3 times for 10 minutes each with TBS-T then blotted with 1:2,500 goat anti-rabbit horseradish peroxidase (HRP) secondary antibody (Millipore, Billerica, MA) for 1 hour. Loading control was the nuclear-specific P84/N5–5E10 (mouse anti-human, GeneTex, Irvine, CA) probed at 1:1,000 in TBS-T overnight, washed 3 times for 10 minutes with TBS-T then blotted with 1:15,000 goat anti-mouse-HRP secondary antibody (Millipore, Billerica, MA) for 1 hour. Blots were visualized using Supersignal West Pico (Thermo Scientific, Rockford, IL) and captured with a Fluorchem SP digital imager (Alpha Innotech, San Leandro, CA).

Statistical Analysis

All experiments were performed with a minimum of three independent experiments. Statistical significance was evaluated between groups using unpaired Student's *t*-test with a significance level of $p<0.05$, using Graphpad Prism 6.

Results

Stabilization and nuclear localization of HIF-1α in SK-N-BE cells during thiamine deficiency

To determine the effect of thiamine deficiency on the stabilization of HIF-1α, nuclear lysates of SK-N-BE cells treated with PTD medium were analyzed by Western blot. Figure 1 demonstrates an increase in nuclear localization of HIF-1α after 1, 2, and 5 days of PTD treatment compared to T30 control. Double banding of HIF-1α was observed after some treatments. Unprocessed HIF-1α is ~95 kDa while the fully post-translationally modified form is ~116 kDa and depending on the treatment type it is common to see either a single or a double band [26].

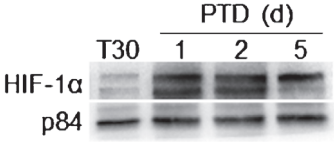

Figure 1: Stabilization and nuclear localization of HIF-1α in SK-N-BE cells during thiamine deficiency. Representative Western blot for HIF-1α in SK-N-BE cells exposed to PTD medium for 1, 2, and 5 days with p84 used as loading control.

Expression of known HIF-1α target genes and thiamine transporters after 1 % O_2 exposure

qRT-PCR was used to determine the effect of 1 % O_2 exposure on known HIF-1α target genes SLC2 A1 (GLUT1), vascular endothelial growth factor (VEGF), and aldolase A. As seen in Figure 2A, expression of GLUT1, VEGF, and aldolase A increased with time, peaking at 5.4-, 18.6-, and 2.9-fold, respectively after 48 hours of 1 % O_2 exposure. The effect of 1 % O_2 exposure on the expression of the thiamine transporters SLC19A2 (THTR1) and SLC19A3 (THTR2) was determined using qRT-PCR. Figure 2B demonstrates no change in SLC19A2 expression over the time course of 1 % O_2 exposure. Alternatively, SLC19A3 expression levels increased after 12 hours of 1 % O_2 treatment and reached ~11.5-fold after 48 hours.

Impact of thiamine deficiency on the expression of known HIF-1α target genes and thiamine transporters

To determine the effect of thiamine deficiency on the expression of GLUT1, VEGF, and aldolase A, mRNA expression levels were quantified using qRT-PCR. GLUT1, VEGF, and aldolase A all showed a time-dependent increase in gene expression reaching ~3.4-, 6.2-, and 3.0-fold, respectively after 5 days of thiamine deficiency (Figure 2C). As shown in Figure 2D, SLC19A2 expression levels showed no change with 1.06-, 1.12-, and 1.04-fold change after 1, 2, and 5 days, respectively. SLC19A3 expression levels increased over time with a fold change of ~0.8, 2.0, and 3.4 after 1, 2, and 5 days, respectively.

Attenuation of HIF-1α response under 1 % O_2 exposure and thiamine deficiency

Western blotting was used to establish whether the HIF-1α inhibitor YC1 decreased HIF-1α protein stabilization and nuclear localization after treatment to

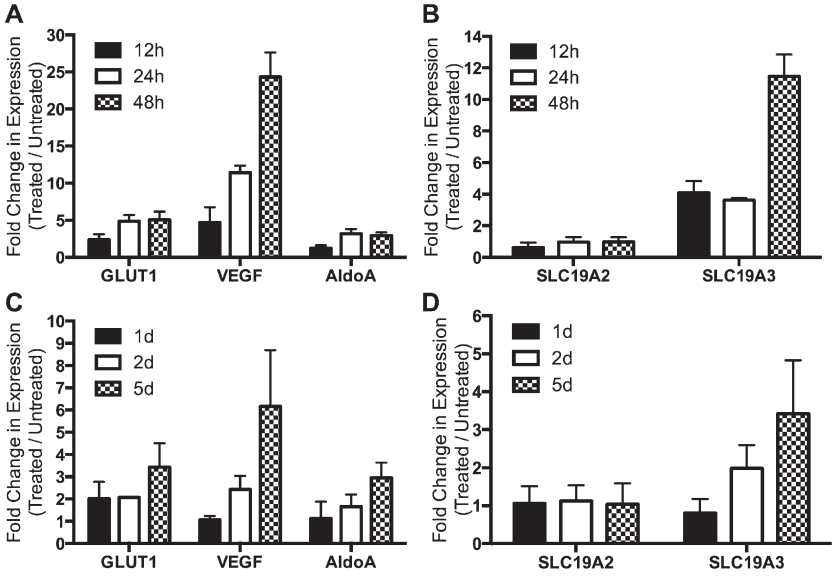

Figure 2: Effect of oxygen and thiamine deprivation on HIF-1α mediated gene expression. Fold change in gene expression for (A) GLUT1, VEGF, and aldolase A and (B) the thiamine transporters SLC19A2 and SLC19A3 in SK-N-BE cells after 1 % O_2 exposure for 12, 24, and 48 hours. Fold change in gene expression for (C) GLUT1, VEGF, and aldolase A and (D) the thiamine transporters SLC19A2 and SLC19A3 in SK-N-BE cells after PTD treatment for 1, 2, and 5 days.

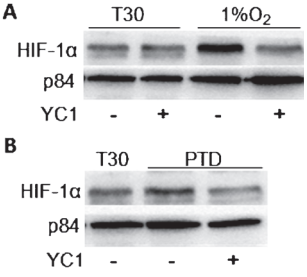

Figure 3: Effect of YC1 on HIF-1α stabilization after 1 % O_2 and PTD exposure. Representative Western blots for the nuclear localization of HIF-1α in SK-N-BE cells grown in T30 media after (A) 1 % O_2 exposure for 48 hours and (B) PTD media for 5 days compared to untreated control cells in the presence and absence of YC1 with p84 as loading control.

SK-N-BE cells. Figure 3A demonstrates HIF-1α stabilization in nuclear samples treated under normoxic and hypoxic conditions for 48 hours, with or without the addition of YC1. Compared to normoxic control cells, the presence of YC1 had no effect on HIF-1α. Alternatively, there was a decrease in HIF-1α stabilization in cells treated with 1 % O_2 with the addition of YC1. Figure 3B shows HIF-1α stabilization in cells treated with PTD with a decrease in HIF-1α expression in cells treated with PTD in addition to YC1, indicating that YC1 treatment causes a reduction in HIF-1α nuclear localization.

YC-1 significantly attenuated the HIF-1α-mediated induction of GLUT1 and VEGF expression under hypoxic conditions, but no significant change was found for aldolase A (Table I). YC1 treatment also significantly attenuated the hypoxia-mediated induction of SLC19A3 (Table I). HIF-1α-DN plasmid transfected into SK-N-BE cells followed by 1 % O_2 for 48 hours significantly attenuated the hypoxia-mediated induction of GLUT1, VEGF, aldolase A, and SLC19A3 (Table I). As shown in Table II, PTD

Table I: Attenuation in the gene expression of GLUT1, VEGF, aldolase A, and the thiamine transporters SLC19A2 and SLC19A3. Cells were treated with or without YC1 or HIF-1α DN in 1% O_2 for 48 hours.

Gene	Untreated	YC1	HIFDN
GLUT1	1.86 ± 0.72	0.66 ± 0.16*	1.22 ± 0.63**
VEGF	23.1 ± 6.61	7.06 ± 1.94*	10.2 ± 4.57 **
Aldolase A	5.24 ± 1.72	2.67 ± 0.15	1.01 ± 0.44**
SLC19A2	0.31 ± 0.19	0.34 ± 0.14	0.46 ± 0.09
SLC19A3	6.54 ± 1.64	2.24 ± 0.81*	2.26 ± 0.37**

Results are expressed as the average fold change ± SD compared to SK-N-BE cells cultured in normoxic conditions (untreated) with n = 3 independent experiments. Statistically significant ($p < 0.05$) comparisons between untreated and YC1 (*), and untreated and HIFDN (**).

Table II: Attenuation in the gene expression of GLUT1, VEGF, aldolase A, and the thiamine transporters SLC19A2 and SLC19A3. Cells were treated with or without YC1 or HIF-1α DN in PTD for 5 days.

Gene	Untreated	YC1	HIFDN
GLUT1	3.96 ± 1.23	1.01 ± 0.21*	0.99 ± 0.78**
VEGF	5.05 ± 2.25	2.36 ± 0.37*	1.89 ± 0.88 **
Aldolase A	2.55 ± 0.53	1.43 ± 0.52*	1.84 ± 1.06
SLC19A2	1.04 ± 0.54	0.79 ± 0.16	0.91 ± 0.51
SLC19A3	3.22 ± 1.73	0.91 ± 0.67*	1.35 ± 0.19**

Results are expressed as the average fold change ± SD compared to SK-N-BE cells cultured in T30 conditions (untreated) with n = 3 independent experiments. Statistically significant ($p<0.05$) comparisons between untreated and YC1 (*), and untreated and HIFDN (**).

treatment for 5 days with YC1 significantly attenuated the induction of gene expression for GLUT1, VEGF, aldolase A, and SLC19A3. SK-N-BE cells transfected with HIF-1α-DN and treated with PTD for 5 days also had a significantly attenuated induction of gene expression for GLUT1, VEGF, and SLC19A3 (Table II).

Impact of thiamine deficiency on pyruvate and lactate production

To establish whether limiting thiamine availability results in an accumulation of pyruvate and lactate, intracellular pyruvate and lactate levels were quantified in cell lysates after exposure to PTD for 1, 2, and 5 days. Figure 4A demonstrates a time dependent increase in cellular pyruvate levels during PTD treatment compared to T30 cells increasing to ~2.5-fold after 5 days. There was no change in intracellular lactate production after 1 and 2 days of PTD treatment as shown in Figure 4B. However, there was a slight increase of ~1.6-fold in intracellular lactate accumulation after day 5 of PTD exposure. Levels of extracellular lactate did not change over 1 and 2 days, but increased ~3-fold after 5 days of PTD treatment relative to T30 (Figure 4C).

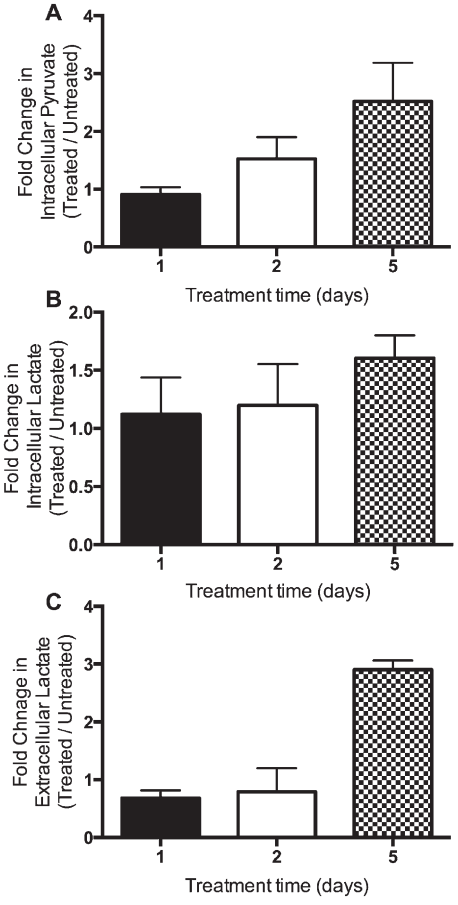

Figure 4: Impact of thiamine deficiency on pyruvate and lactate levels. The fold change in the intracellular levels of (A) pyruvate and (B) lactate and (C) extracellular lactate levels after exposure to PTD for 1, 2, and 5 days. Results are expressed as the average fold change ± standard deviation compared to SK-N-BE cells cultured under T30 conditions with n = 3 independent experiments.

Discussion

Hypoxia/ischemia is associated with a number of pathologies including cancer, diabetes, Alzheimer's disease, and cardio/cerebrovascular disease [27–29]. HIF-1α is a well characterized transcription factor that plays a central role in the cellular adaptation to low oxygen, mediating a reprogramming of glycolytic metabolism [30]. Both hypoxia and TD are associated with decreased ATP, increased cellular glycolysis, lactate secretion, and tissue acidosis [31–33]. This

metabolic congruency may suggest a common adaptive response that shifts towards a metabolic phenotype with a reduced need for thiamine and oxygen.

Our findings that TD increases HIF-1α stabilization and gene expression suggest the metabolic consequences of TD may centralize with HIF-1α mediated metabolic reprogramming. HIF-1α induces the expression of the glucose transporter GLUT1, resulting in an increase in glucose consumption under hypoxic stress [34]. An increase in glucose consumption was found in pyruvate dehydrogenase (PDH)-deficient fibroblast cells compared to normal cells, demonstrating an increased reliance on glycolytic metabolism when devoid of this thiamine-dependent enzyme [35]. Our findings also demonstrated an increase in expression of GLUT1 during TD that may be a contributor for increasing glucose consumption. TD increased expression of the HIF-1α target gene aldolase A, a glycolytic enzyme that converts fructose 1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone. The increased expression of aldolase A may assist in increasing glycolysis to maximize ATP generation. HIF-1α also induces angiogenesis through the increased expression of VEGF in an attempt to increase vascular density and supply of oxygen [35]. Both hypoxia and TD induced VEGF expression in SK-N-BE cells. Although it is unknown if TD is associated with angiogenesis, increases in pyruvate have demonstrated angiogenic activity *in vitro* and *in vivo* [37]. Thus, the resulting effects of TD on cellular metabolism and subsequent sequelae associated with TD disorders may be mediated through HIF-1α effects on metabolic reprogramming.

When thiamine availability is limited, an adaptive transcriptional up-regulation of the thiamine transporter SLC19A3 has been observed in intestinal and renal tissues in an attempt to increase thiamine absorption and reduce clearance [38,39]. Consistent with these findings we observed that TD treatment of SK-N-BE cells resulted in an up-regulation of SLC19A3 expression with no change in SLC19A2. We have previously demonstrated the up-regulation of SLC19A3 and an increase in thiamine transport during hypoxic exposure to breast cancer cells that was reduced by HIF-1α shRNA [40]. Synonymous to these findings, hypoxia increased SLC19A3 in SK-N-BE cells and was attenuated by the HIF-1α inhibitor YC1 and the dominant negative form of HIF-1α in TD and hypoxia treatments. This further demonstrates a potential regulatory role of HIF-1α in mediating thiamine transporter expression during hypoxic and nutritional stress.

Although stabilization of HIF-1α is most commonly associated with low oxygen tensions, reduced degradation and induction of HIF-1α-mediated gene expression are also associated with the accumulation of metabolic intermediates independent of oxygen. In particular, increases in cellular pyruvate and lactate have been shown to stabilize HIF-1α and mediate gene expression [14,41]. Pyruvate has been suggested to stabilize HIF-1α through inhibition of HIF-1α degradation, however the exact mechanism remains undetermined [14]. Pyruvate is formed within the Embden-Meyerhof pathway and is converted to acetyl-CoA by PDH. Reduced expression of PDH has been reported under a state of TD indicating the potential role of thiamine in regulating the activity of this thiamine-dependent enzyme [42]. It has been suggested that activity of α-KGDH is reduced prior to the onset of pathologic lesions while PDH activities is only reduced at later stages [43]. However, only changes in α-ketoglutarate dehydrogenase (α-KGDH) and not PDH activity has been typically reported in whole brain homogenate of thiamine-deficient animals [7,44]. Thiamine deficiency has been associated with inducing region-specific lesions in the brain and as such changes in enzyme activity within whole brain homogenate may not represent regions affected by TD [9,45]. While no reports to date have demonstrated direct increases in pyruvate levels within the whole brain of TD animals, a decrease in PDH activity and reduced pyruvate flux within nerve terminals of the forebrain in TD rats was recently reported [9].

When in excess, pyruvate can be interconverted to lactate by lactate dehydrogenase [46,47]. An increase in lactate production is an indicator of HIF-1α-mediated glycolytic shift and a common clinical sign of thiamine deficiency [48]. Our findings demonstrated that TD increased the extracellular secretion of lactate, consistent with the metabolic impact of TD and hypoxia. Although lactate has been reported to stimulate HIF-1α protein accumulation, it is suggested that lactate requires the conversion to pyruvate to induce pseudo-hypoxia [14]. However, it is unclear if the increase in pyruvate is due to inter-conversion from lactate or a combination of the reduction in the functionality of other thiamine-dependent enzymes.

In conclusion, our results indicate TD induces HIF-1α-mediated gene expression similar to that observed during hypoxic stress. These results are the first to demonstrate a potential transcriptional congruency between hypoxia and TD that may explain the clinical manifestations occurring in pathologies associated with thiamine deficiency. In addition, the potential role of HIF-1α in thiamine transporter expression may provide new insights into the adaptive regulation during hypoxic and nutritional stress.

Acknowledgements

Research reported in this publication was supported by the National Institute On Alcohol Abuse And Alcoholism of the National Institutes of Health under Award Number R21AA021948.

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