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Impact of Prevailing Thiamin Levels on Thiamin Pyrophosphate Uptake in Pancreatic Acinar Cells: Do The Shuttle!

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Abbreviations:

ATP, adenosine triphosphate; MCF, mitochondrial carrier family; MTPPT, mitochondrial thiamin pyrophosphate transporter; NF-Y, nuclear factor-Y; PAC, pancreatic acinar cells; thiamin-DEF, thiamin-deficient; thiamin-OS, thiamin-over-supplemented; THTR, thiamin transporter; TPP, thiamin pyrophosphate

Thiamin is a water-soluble B1 vitamin that regulates critical cellular processes, such as oxidative energy metabolism, adenosine triphosphate (ATP) production and mitochondrial function; thus, it is referred to as the energy vitamin (1, 2). Based on these known roles of thiamin, deficiency of this vitamin can lead to serious cellular impairments, including reduced cellular energy, increased oxidative stress and mitochondrial dysfunction (3).

Pancreatic acinar cells (PAC) maintain a high degree of metabolic activity, and therefore require larger amounts of thiamin (4); however, PAC are unable to synthesize their own thiamin and thus require thiamin via dietary or supplemental intake (5). Thiamin is taken up by the cell through thiamin transporter (THTR)-1 and -2, is largely converted (80-90% of thiamin) into thiamin pyrophosphate (TPP), and is then transported into the mitochondria by the mitochondrial TPP transporter (MTPPT) (6). The MTPPT, encoded by the *SLC25A19* gene, is a member of the mitochondrial carrier family (MCF), and previous work has shown that *SLC25A19* promoter activity is driven by nuclear factor (NF)-Y function (7). Additionally, it has been shown that chronic exposure to alcohol and cigarette smoke affect mitochondrial TPP uptake in PAC cells (8, 9); however, it is unknown if extracellular thiamin levels affect this process as well.

The function of the MTPPT shuttle is of clinical relevance since mutations in the transporter are linked to Amish congenital lethal microcephaly, as well as neuropathy and bilateral striatal necrosis (10, 11). Based on this information, this study by Sabui et al. aimed to determine the effect of the prevailing level of thiamin on mitochondrial TPP

uptake by PAC cells, using both PAC 266-6 (cultured PAC) and transgenic mice carrying the human *SLC25A19* promoter (12).

In this study, the effects of thiamin levels on mitochondrial [^{3}H]-TPP uptake was first shown in PAC maintained in either thiamin-deficient (thiamin-DEF) or over-supplemented (thiamin-OS) conditions (12). These initial results indicate that mitochondrial [^{3}H]-TPP uptake was increased in PAC 266-6 maintained in thiamin-DEF conditions as compared to thiamin-OS conditions. To corroborate these findings in an *in vivo* model, the authors fed mice either a thiamin-DEF or thiamin-OS diet and evaluated mitochondrial TPP uptake in hepatocytes. The authors argue that the use of hepatocytes for these studies is valid since (i) it is difficult to obtain a sufficient concentration of PAC mitochondria for studies, (ii) PAC and hepatocytes share the same embryonic lineage, and (iii) this method has been utilized by other researchers (13, 14). Similar to the *in vitro* study, the authors found that mitochondrial [^{3}H]-TPP uptake was increased in hepatocytes isolated from mice fed a thiamin-DEF diet versus mice fed a thiamin-OS diet. These findings are the first to indicate that prevailing thiamin levels regulate TPP shuttling in PAC. While the use of hepatocytes for these studies is experimentally valid, it may be beneficial in the future to look at isolated PAC; however, this may be dependent on the development of a method that isolates a larger concentration of PAC.

As previously stated, thiamin regulates ATP production; therefore, the authors evaluated the effect of extracellular thiamin levels on PAC 266-6 ATP production. Using the same *in vitro* studies as described above, the authors found that PAC 266-6

maintained in thiamin-DEF conditions had a significant reduction in ATP levels when compared to cells maintained in thiamin-OS conditions. Considering TPP taken up by the mitochondria acts as a co-factor for pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and branched-chain α-keto acid dehydrogenase, it is not surprising that thiamin deficiency leads to a decrease in ATP production (11, 15).

Since mitochondrial TPP uptake is regulated by the MTPPT, the authors examined the expression of MTPPT in PAC 266-6 maintained in either thiamin-DEF or thiamin-OS conditions. The results showed that PAC 266-6 cultured in thiamin-DEF conditions have a significant increase in both mRNA and protein levels of MTPPT when compared to thiamin-OS conditions. These findings were corroborated *in vivo* where the authors noted an increase in both mRNA and protein levels of MTPPT in hepatocytes isolated from mice fed a thiamin-DEF diet versus mice fed a thiamin-OS diet. These findings indicate that the increase in TPP uptake, in response to a thiamin-DEF surrounding, may be dependent on changes in expression of the MTPPT.

Based on the fact that the levels of MTPPT mRNA expression increase in response to increased thiamin levels, the authors suggest that this may be mediated by transcriptional changes in *SLC25A19*. Similar mechanisms have been shown to mediate the adaptive-regulation of a multitude of other water-soluble vitamins (16). To evaluate this hypothesis, the authors evaluated the activity of the human *SLC25A19* promoter (transfected into PAC 266-6 cells) in both thiamin-DEF and thiamin-OS conditions. The authors found that *SCL25A19* activity was increased in PAC 266-6 cultured in thiamin-DEF conditions versus thiamin-OS conditions. To relate these findings to an *in vivo*

model, the authors utilized their transgenic mice carrying the human *SLC25A19* promoter (fused with *firefly* luciferase reporter gene), which has previously been characterized by this group (8), fed either a thiamin-DEF or thiamin-OS diet. Results from this study demonstrated that *SLC25A19* activity is increased in hepatocytes isolated from transgenic mice fed a thiamin-DEF diet versus a thiamin-OS diet. These results suggest that PAC mitochondrial TPP uptake is dependent on adaptive-mediated transcriptional changes in *SLC25A19*, thereby affecting MTPPT expression. Additionally, the use of a transgenic mouse model expressing the human *SLC25A19* gene is novel and brings about additional human relevance to the study.

Transcriptional changes in the *SLC25A19* gene may be mediated by either (i) nuclear factors that regulate basal activity of the promoter and/or (ii) epigenetic changes, such as histone modifications. To evaluate whether nuclear factors were regulating *SCL25A19* promoter activity, PAC 266-6 were maintained in either thiamin-DEF or thiamin-OS conditions and the expression of NF-Y (a transcription factor known to regulate MTPPT expression) was evaluated. Results from this study found that there was no change in NF-Y mRNA levels in PAC 266-6 cultured in either condition. The authors then hypothesized that NF-Y may exhibit changes in binding affinity to the *SLC25A19* promoter dependent on culture conditions. The authors found that NF-Y binding to the *SLC25A19* promoter was significantly increased in PAC 266-6 cultured in thiamin-DEF conditions compared to thiamin-OS.

As previously discussed, epigenetic changes, such as histone modifications, may play an important role in the regulation of the expression of the *SLC25A19* gene (8).

Through *in vitro* analysis, the authors found that there was a significant increase in the euchromatin (activating) markers H3K4me3 and H3K9Ac in PAC 266-6 cultured in thiamin-DEF conditions versus thiamin-OS conditions; however, expression of the heterochromatin (repressing) marker H3K27me3 was significantly decreased in PAC 266-6 cultured in thiamin-DEF conditions versus thiamin-OS conditions. Overall, these findings allude to the role that thiamin levels may have on histone modification of the *SLC25A19* gene, thereby regulating MTPPT uptake in PAC.

Little is known about how the TPP shuttling process is regulated in PAC; therefore, the findings of this study are noteworthy. This study is the first to identify that extracellular thiamin levels regulate mitochondrial TPP uptake and further expand the knowledge of the field. Additionally, the authors recognize that changes in uptake are dependent on transcriptional and epigenetic changes occurring in the *SLC25A19* gene (encodes MTPPT). While it is interesting to note that both transcriptional and epigenetic changes are regulating MTPPT availability, these findings were from *in vitro* experimentation; therefore, additional work in *in vivo* models is necessary. Furthermore, these findings were in the context of normal PAC exposed to either thiamin-DEF or thiamin-OS conditions. It is important to understand if the changes noted are also occurring during diseased states. Since the authors have previously published data indicating that chronic exposure of PAC to alcohol negatively impacts MTPPT function through transcriptional and epigenetic changes (8), there may be a novel field for the development of therapeutics but further work is necessary. Furthermore, while the authors analyzed the molecular changes mediating mitochondrial TPP shuttling they did

not evaluate whether the changes in TPP uptake influenced the activity and/or viability of the cell. Since TPP is known to regulate oxidative stress and ATP production, which was reduced during thiamin-DEF conditions, it is intuitive that changes in these processes may impede cellular function.

Based on their findings, the authors come to the overall conclusion that PAC mitochondrial TPP uptake is adaptively regulated by extracellular thiamin levels, and this mechanism is partially regulated by both transcriptional and epigenetic changes in the *SLC25A19* promoter. While this manuscript is highly novel and presents interesting findings, there are more questions that need answering. In the future, it is imperative that additional work be performed to help completely elucidate the role of thiamin deficiency on MTPPT activity.

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