Effects of Environmental Conditions on Thiamine Levels of Brook Trout

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Introduction

How do Thiamine levels affect Brook Trout?

- **Brook trout (Salvelinus fontinalis)**, a predatory freshwater species, often consume prey fish with elevated levels of thiaminase.[1,5] Previous studies have shown that **thiaminase degrades thiamine** (Vitamin B1), an essential **cofactor** in metabolic pathways including the pentose-phosphate pathway, citric acid cycle, and glycolysis. This study investigated the impact of environmental factors on the thiamine levels in the diets of these brook trouts.
- Thiamine is found in three forms in the body, with Thiamine Diphosphate (TDP) comprising about **80% of the total Thiamine concentration**.[4,5]
- Deficiencies in TDP are attributed to **cardiac and central nervous system impairments**, such as beriberi and Wernicke–Korsakoff syndrome respectively, as well as the **impairment of RNA and DNA synthesis** due to reduced NADPH production.[1,4,5]

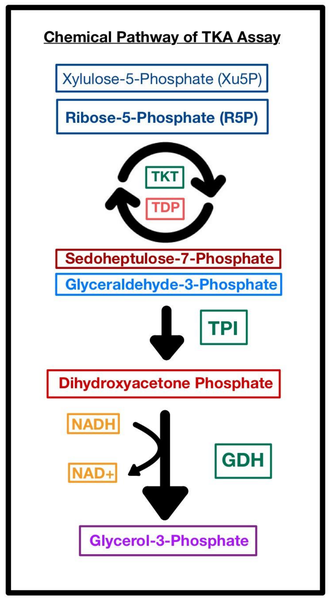

Fig. 1: This figure displays the TKA assay's chemical pathway, and how the NADH absorbance is measured via oxidation

What is Transketolase?

- **Transketolase** (TKA) is an important enzyme in the non-oxidative phase of the Pentose-Phosphate Pathway that reformulates sugars for glycolysis, and downstream creation of NADH and NAD+ byproducts.[1,2,3]
- **TDP is an essential cofactor for TKA**, and is required to maintain a balance in cellular redox states.[2,3]
- Since the Pentose-Phosphate Pathway has a multitude of cellular reactions, the simplified portion that includes TKA and the chemical pathway for our assay is shown on the figure to the left.[2,3]
- In this study, TKA is the primary enzyme being analyzed, and is observed to see its **endogenous (base) and stimulated (added) activity**.[3]

What is a Kinetic Enzyme Assay?

- A kinetic enzyme assay is used to **analyze the activity of an enzyme** by continuously measuring its rate of reaction, and the concentration of products or substrates over time.[6]
- This can be done by creating a downsized biochemical reaction and then measuring the peak absorbances of the products or substrates over time.[2,3,6]

Research Questions & Hypothesis

1) **To what extent does a deficiency in thiamine affect cellular redox reactions?**
2) **How can thiamine concentrations be efficiently determined with kinetic enzyme assays?**
3) **What do our assay absorbance values tell us about the metabolic processes of Brook Trout?**

Hypothesis: When TDP is added to a homogenized Brook Trout liver sample with proper reagents, its absorbance of NADH will decrease over time more so than the samples without added TDP. The differences in slopes of absorbance can be used to determine deficiency in thiamine levels.

Methods

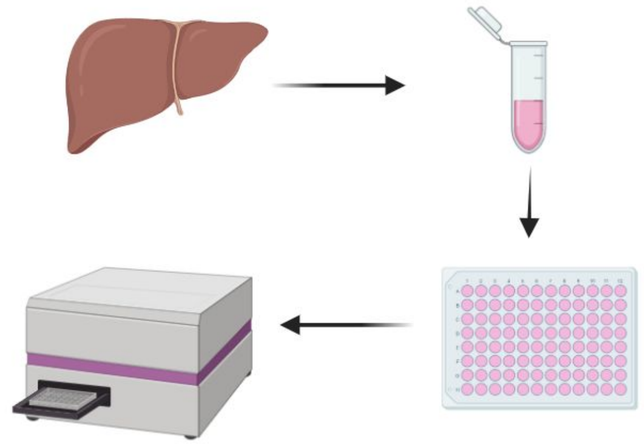

***Fig. 2*: Measuring NADH concentration from homogenized fish liver in sucrose**

Results

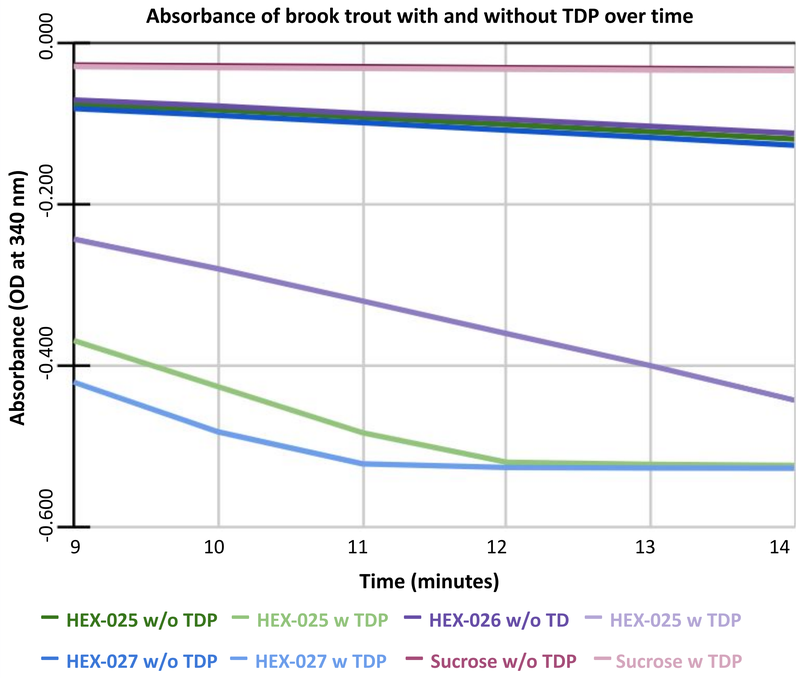

***Fig. 3*: Measured absorbance read at 340 nm of NADH in brook trout samples with and without added TDP over time.** Metabolic activity in livers taken from brook trout samples were analyzed through a kinetic enzyme assay. Both the endogenous (without TDP) and stimulated (with added TDP) activity were observed by measuring absorbance at 340 nm. Between minutes 9 and 14, samples with TDP decreased in absorbance at a greater rate than the same samples without TDP, indicating the TDP lead to faster conversions of NADH to NAD+. Data obtained was used to calculate EKTA values for each sample. Sucrose was used as a control.

Results

Table 1: Samples with EKTA < 1.000 display deficiency in thiamine

Brook Trout Sample	EKTA Value
HEX-025	3.609
HEX-026	4.835
HEX-027	2.107
HEX-028	7.085
HEX-029	0.355
HEX-035	1.297
HEX-036	0.031
HEX-037	3.636
HEX-038	0.132
HEX-039	1.050
HEX-050	1.032
HEX-051	4.053
HEX-052	0.494
HEX-053	0.543
HEX-054	0.058
Sucrose	0.925

Conclusion

- In samples with sufficient thiamine levels, adding TDP caused a faster decrease in NADH absorbance from minutes 9 to 14 than in the same samples without added TDP
- Samples HEX-029, HEX-038, HEX-052, HEX-053, and HEX-054 have a deficiency in thiamine as indicated by their erythrocyte transketolase activity (EKTA) being less than 1.000
- A transketolase kinetic enzyme assay can be used to determine how the environments of fish affects their thiamine levels and overall health

Future Works

Other studies that could be further conducted:

Regulated Enzymes:

- Can other enzymes, such as glucose dehydrogenase (GDH), be directly measured via a similar kinetic enzyme assay?

Cellular Redox States:

- Can molecules, such as NAD+ or NADPH, be measured rather than NADH?

Oxidative Stress:

- How do thiamine levels and transketolase activity differ between fish in hypoxic and non-hypoxic conditions?

Acknowledgments & References

Thank you to our Research Educator, Dr. Katie Edwards, Ph.D., for her continuous support and guidance throughout our research project.