Effects of methionine restriction and carbohydrate-rich diets on middle intestinal cells of *Oncorhynchus mykiss*

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Introduction

While the phenotypic impacts of methionine-restricted (MR) diets has been the subject of many recent metabolic studies, by raising rainbow trout (*Ocorhynchus mykiss)* on a 12% (nominal) and a 22% (high) carbohydrate diet, with and without MR, Craig & Moon (2012) have recently shown that such effects are not limited to mammalian species.

Fig 1: Diets restricted in methionine have been shown to have significant impact on the phenotypes of mammalian species. Studies report "extended life span, delayed onset of age-related diseases and enhanced fat oxidation in obese subjects" (Craig & Moon, 2012).

Craig & Moon (2012) observed that rainbow trout, an otherwise glucose intolerant species, fed a diet restricted in methionine abolished the glucose intolerant phenotype 6 hours after feeding as well as alter their levels of stored liver carbohydrate.

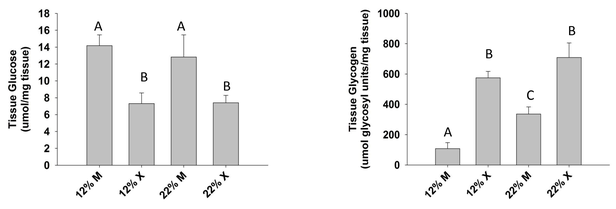
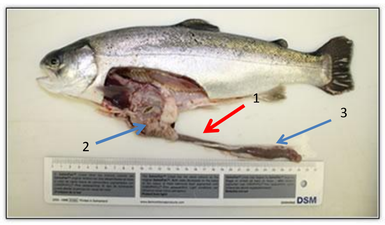


Fig 2: Glucose and glycogen levels in the livers of rainbow trout fed a diet of either 12% or 22% carbohydrate, with (M) or without (X) methionine restriction. A substantial decrease in tissue glucose was observed under the (X) conditions while an substantial increase in tissue glycogen was observed with 22% carbohydrate and the (X) condition.

The focus of this study was to investigate whether MR had similar effects on intracellular glucose and glycogen levels of the middle intestine, as well as to assess the activity level of the enzymes superoxide dismutase (SOD) and catalase. Since both enzymes are involved in the conversion of reactive oxygen species, produced during energy metabolism, into H_2O and O_2 their activities serves as markers for the metabolic activity of the cells as a whole.

Fig 3: Gastrointestinal tract of a rainbow trout. The middle intestine is seen here as the narrow-most portion of the elongated intestine (1). The middle intestine was chosen for study as it possesses intermediate absorptive and secretory activity relative to the anterior (2) and posterior (3) sections, respectively.



Superoxide Dismutase & Catalase Assay

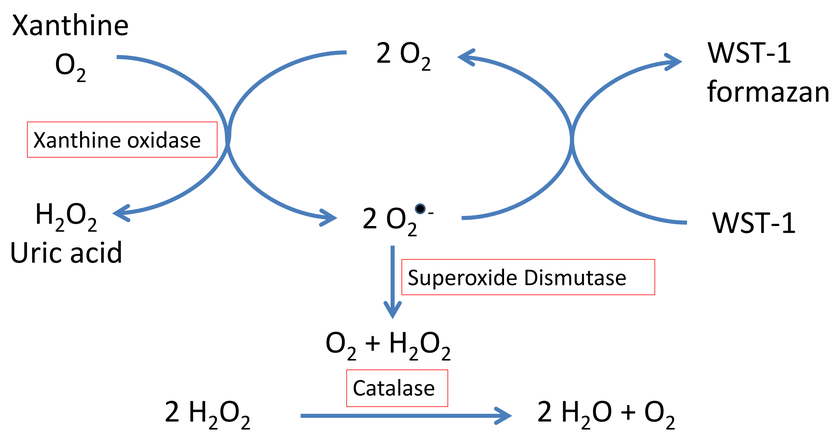


Fig 5: Reaction pathways used to assess the activity levels of the enzymes superoxide dismutase and catalase. SOD activity was calculated based on the 50% inhibition of xanthine oxidase as measured spectrophotmetrically at 440nm, corresponding to the absorbance of formazan, while catalase activity was measured on the rate of H_2O_2 consumption, as monitored by spectrophotometry at 240 nm.

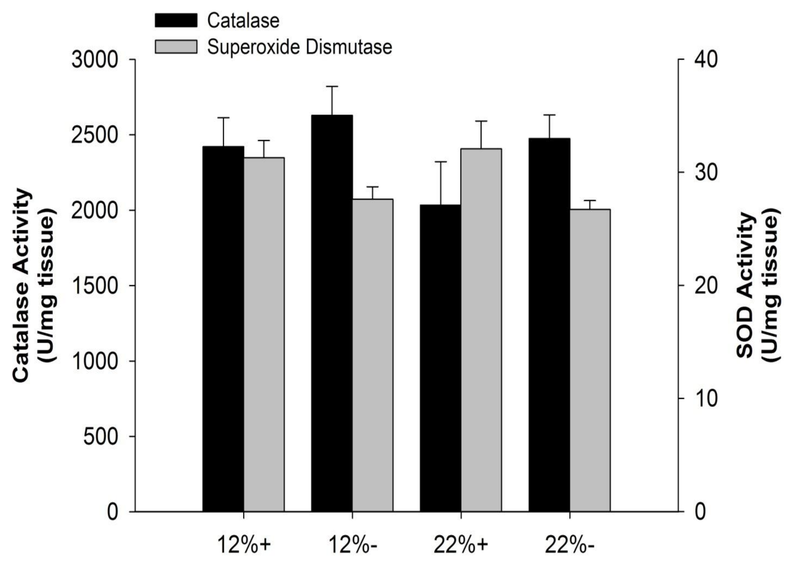


Fig 6: Activity levels of the enzymes catalase and superoxide dismutase in the middle intestinal cells of rainbow trout (n=6/group) fed diets of 12% and 22% carbohydrate, with and without methionine (+/-). Enzymatic activity was assayed using commercially available kits from Sigma-Aldrich Inc. (St. Louis, MO, USA). No significant difference in the activity of each enzyme was noted across all groups.

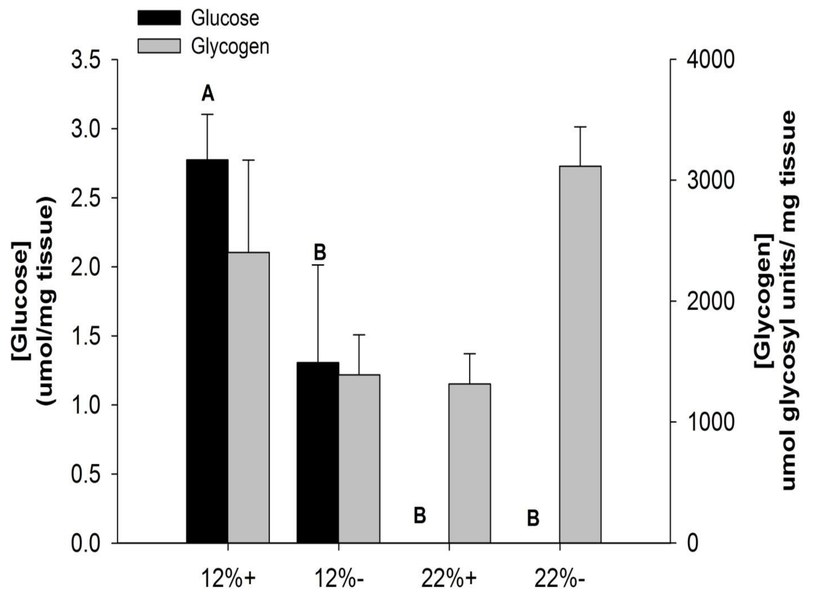


Fig 8: Glucose and glycogen concentrations per mg of tissue in the middle intestinal cells of rainbow trout (n=6/group) fed diets of 12% and 22% carbohydrate, with and without methionine (+/-). The 12%+ group showed a significant increase in glucose levels compared to all other groups. No significant difference in glycogen levels was observed across all experimental groups.

Conclusion

Being that both SOD and catalase are involved in the metabolic pathways that processes harmful oxygen free radicals, it appears that both carbohydrate load and the presence or absence of methionine have no effect on the energetic processes which yield reactive oxygen species. From this we can infer that the oxygen consumption, therefore metabolic rate, of rainbow trout middle intestinal cells remains relatively unaffected regardless of carbohydrate intake or MR.

Statistical analysis showed a significant increase in glucose levels within the 12%(+) group (A) in comparison to all others (B). This is contrary to our hypothesis as we would expect the amount of free glucose to correlate positively with the amount of carbohydrate present in the diet and thus expect to see higher levels in the 22% groups. It could be suggested that the intestinal cells are able to take up glucose when carbohydrates levels are within a nominal range unless the amount of dietary methionine is restricted. Under significantly higher hyperglycemic conditions, such as the 22% carbohydrate diet, the intestinal cells are either unable to efficiently take up glucose, or rapidly transport glucose from the intestinal cells.

Despite these findings, the precise relationship between methionine restriction and glucose intolerance in aquatic species warrants further study. In particular, the anterior and posterior intestine should be examined to see how the results of this investigation compare to the gastrointestinal tract of rainbow trout as a whole.

Acknowledgements

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References

Craig PM & Moon TW (2012). Methionine-restriction affects the phenotypic and transcriptional response of rainbow trout (*Oncorhynchus mykiss*) to carbohydrate-enriched diets. British Journal of Nutrition *(In revision*).

Bergmeyer HU (1983) Methods of Enzymatic Analysis. New York, Academic Press.

Methods & Results

Middle intestinal tissue samples were obtained from rainbow trout fed 12% and 22% carbohydrate diets, with and without the amino acid methionine (+/-) for 8 weeks. All tissue samples were powdered under liquid nitrogen using a pestle and mortar then used to carry out the various assays of the investigation.

	12% (+)	12% (-)	22% (+)	22% (-)
Ingredient	**(Kcal/Kg)**	**(Kcal/Kg)**	**(Kcal/Kg)**	**(Kcal/Kg)**
L-Methionine	**56**	**0**	**56**	**0**
L-Alanine	**130.4**	**163.8**	**130.4**	**163.8**
Other Free AA	1864.8	1864.8	1864.8	1864.8
Soybean oil	450	461.25	270	281.25
Menhaden oil	450	461.25	270	281.25
Dextrin	**226.875**	**226.875**	**402.93**	**402.93**
Dyetrose	203.3	203.3	387.6	387.6
Gelatin	284.8	284.8	284.8	284.8
Vitamin Mix	24	24	24	24
Total (Kcal/kg)	**3690.18**	**3690.08**	**3690.53**	**3690.43**

Fig 4: Caloric distribution of rainbow trout diets, with (+) and without (-) methionine restriction, consisting of either 12% or 22% carbohydrate (dextrin). The amounts of all other nutrients were adjusted to ensure that the number of calories consumed in each group were approximately equivalent regardless of the experimental group. The dietary alanine content was also adjusted to ensure the diets were iso-nitrogenous. Diets were purchased from Dyets Inc. (Bethlehem, PA, USA).

Glycogen Assay

The glucose and glycogen content of the intestinal tissue samples were extracted through a series of homogenization and centrifugation steps yielding a supernatant containing the relevant molecules. Two aliquots were taken from each sample; one to assess the free glucose content and another to assess the total glucose content after glycogen digestion. The amount of glucose was measured according to protocol outlined by Bergmeyer (1983).

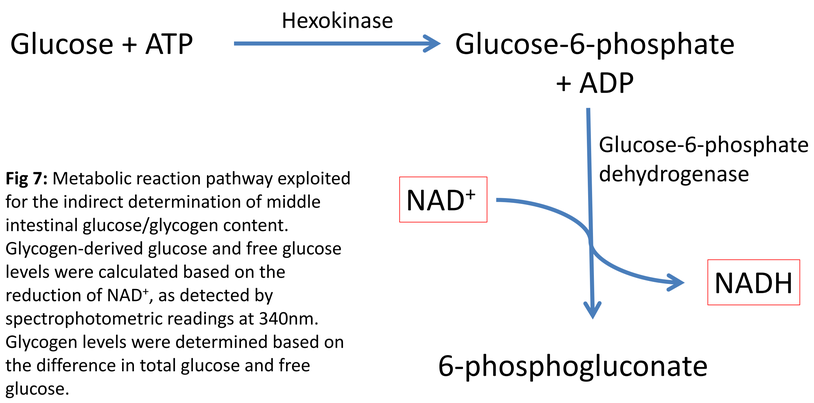

Fig 7: Metabolic reaction pathway exploited for the indirect determination of middle intestinal glucose/glycogen content. Glycogen-derived glucose and free glucose levels were calculated based on the reduction of NAD^+, as detected by spectrophotometric readings at 340nm. Glycogen levels were determined based on the difference in total glucose and free glucose.