Carotenoids protect against oxidative inactivation of an iron-regulatory protein in the marine copepod *Tigriopus californicus*

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Carotenoids are red, orange, and yellow pigments that are the source of coloration in many animals. Carotenoids are thought to have many physiological roles such as immune function and vitamin A synthesis. Carotenoids have been convincingly demonstrated to act as antioxidants in vitro, but evidence for their in vivo antioxidant activity is contentious. In this study, we used the marine copepod Tigriopus californicus to test the antioxidant capacity of carotenoids.

In the wild, T. californicus obtains carotenoids by consuming algae. However, T. californicus can be cultured on yeast, which lack carotenoids. Copepods raised on yeast have small amounts of carotenoids. However, by raising these carotenoid-free yeast copepods in a carotenoid solution, carotenoids can be redeposited into their integument.

We used tert-butyl hydroperoxide (tBHP) as our oxidative stressor. We exposed 320 yeast-fed and carotenoid-restored copepods to either 0 mM or 1 mM tBHP for one hour and then tested for oxidative damage by measuring total aconitase activity (Fig. 1). Aconitase is a protein that contains an iron-sulfur cluster that is sensitive to oxidative damage. In the presence of reactive oxygen species, ferrous (Fe3+) iron will be reduced to ferric iron (Fe2+), causing aconitase inactivation.

Under normal conditions yeast-fed copepods and carotenoid-restored copepods had equal aconitase activity (Yeast: 78.5 ± 10.9, Carot: 80.9 ± 5.1, n = 3, t = -0.293, p = 0.78). tBHP exposure decreased aconitase activity in carotenoid-restored copepods by 6% (Carot-tBHP: 75.83 ± 10.26, n = 3, t = -0.624, p = 0.55), whereas yeast-fed copepods exposed to tBHP had a 34% decrease in aconitase activity relative to unexposed yeast-fed copepods (Yeast-tBHP: 51.5 ± 11.7, Yeast: 78.5 ± 10.9, n = 3, t = -3.35, p = 0.01).

We tested for the functional role of carotenoids as antioxidants by exposing carotenoid-deficient and carotenoid-supplemented copepods to an oxidative challenge and found that carotenoids act to protect against inactivation of a critical metabolic enzyme. Our results corroborate in vitro studies on the antioxidant activity of carotenoids and provide support for a biologically relevant role of carotenoid accumulation as a protectant against oxidative stress in the wild. Future research will incorporate the same carotenoid-rich/carotenoid-free copepod model to investigate other physiological attributes typically associated with increased carotenoid intake.

Statement of Research Advisor:

Philip worked as an independent investigator on the study of the role of carotenoids in protection from oxidative damage in copepods. He designed the study, confronted the numerous problems that arose in the execution of the research, and recently submitted this research to a peer-reviewed journal as a second author–Geoffrey Hill, Biological Sciences

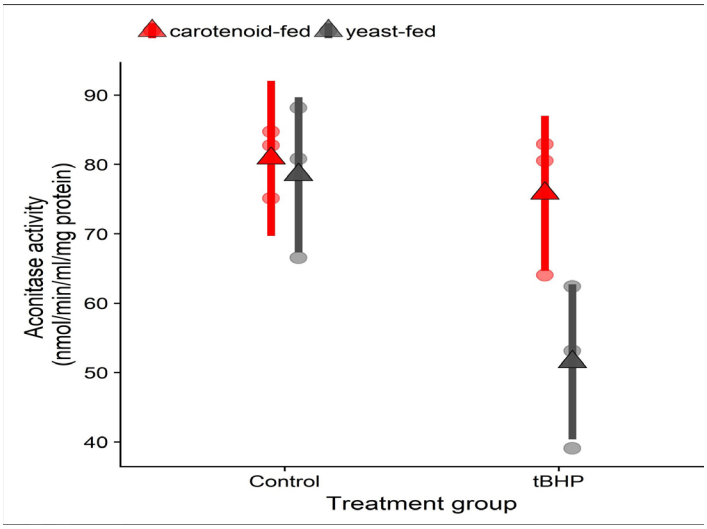

Figure caption: Aconitase activity of carotenoid supplemented (red triangles) and carotenoid deficient (gray triangles) copepods exposed to tBHP versus control.