Effects of Creatine Monohydrate on Zebrafish Embryos

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Abstract

In the module, the purpose of observing the zebrafish with different concentrations of creatine monohydrate was to see the effects this enhancing supplement had on the development behaviors of the fish. During the course of our experiment we found that the zebrafish that were introduced to a dosage of .1g/20ml developed faster than those in the controlled well. The significance of this experiment is to compare the change in the development process of the zebrafish to the changes in humans.

Introduction

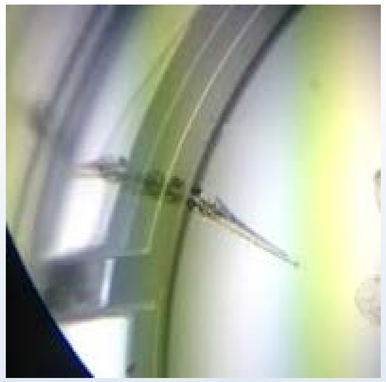
How does the exposure of creatine monohydrate to zebrafish embryos affect behavior and the development? As humans we can obtain natural forms of creatine through eating meat and fish. Carnivores, like wolves, ingest larger amounts of creatine through their diets. Females produce small amounts of creatine in breast milk which is one of the main factors which help babies´ muscle function. Creatine is a supplement made of three amino acids: arginine, glycine and methionine which help synthesize proteins and enhance muscle recovery. In studies done at the Memorial University of Newfoundland, in Canada, researchers found that creatine supplementation can help reduce hepatic steatosis (fatty-liver disease) by reducing the amount of bacteria in the small intestine as well as cleaning the gastrointestinal tract and liver (Brosnan, 2016). The zebrafish have similar development to humans, making them prime targets for the testing of this performance enhancing supplement. Although creatine has evidence supporting the enhancement of muscle development, a study on amino acids in the body done in the UK shows that not all individuals respond to the supplement, as a variety of factors including muscle fiber type, age, and diet can influence the effectiveness of the product (Twycross-Lewis, 2016). Another study done by researchers in Canada shows that creatine can have different effects on different genders, having more of an effect on untrained aging males compared to untrained aging females (Johannsmeier, 2016). In a article ,“A red flag, however, was raised by studies examining rat fetal development. A region of the placenta known as the decidua was found in rodents to express very high levels of the enzyme, AGAT”. “A similar concern arose from the finding that the expression level of the creatine transporter changes dramatically during the first few weeks of life in certain animal species. For instance, in rabbit pups the level of creatine transporter expressed on neurons within the brain drops by 60% between the first 5 to 15 days of life.”

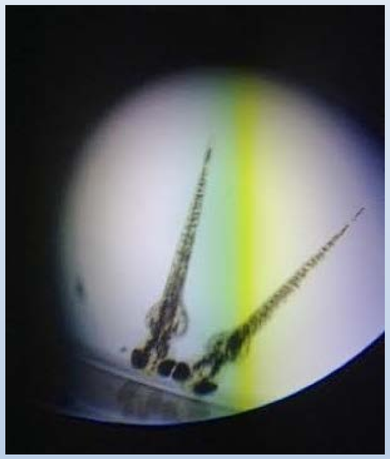
Hypothesis- It is hypothesized that the larger the quantity of creatine monohydrate added to the water the zebrafish embryos are in, the faster the muscles and other organs will develop, increasing the overall rate of development and size.

Materials & Methods

-Add 3 ml of water per well
-concentrations (mix concentrations with 20 ml of embryo media in beaker - initial add 3 ml each day).
-Mix concentrations with different levels of creatine (.1g, .18g, .25g)
-Put 3 ml of baseline solution in 2 wells and 3 ml of each creatine solution into 2 wells each.
-Add 5 zebrafish embryos to each of the 8 wells
-Change the 3 ml of each solution in each well every day
-8 wells 2 control, 2 of each concentration (3 different concentrations)
-Add 5 zebrafish embryos to each of the 8 wells
-Incubate every night (28 C)
-Take pictures and record development of zebrafish each day to show changes in growth
-Compare photos and embryos to development stage s to see progress

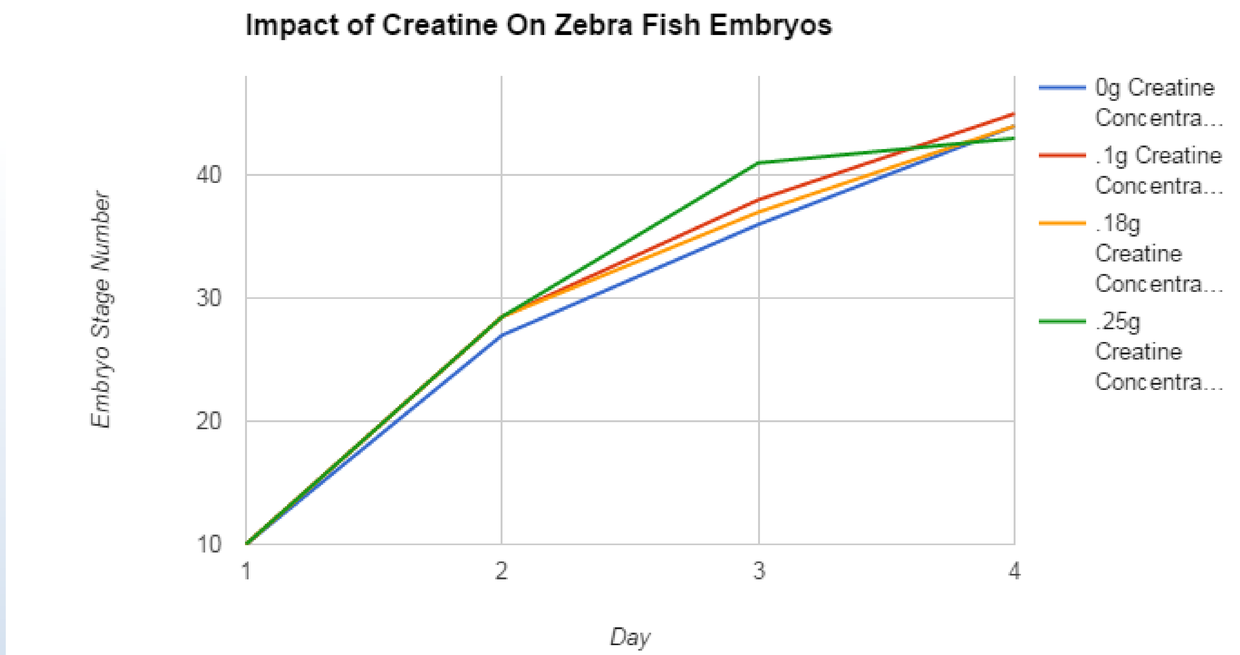
Results





(Bottom Left) An image of some zebrafish that hatched in the .1g solution. Here we can see significant developmental differences to the baseline zebrafish in day 3 of the experiment. (Top Right)

(Below) This chart show the embryo development stage of the zebrafish along a 4 day period. Our results showed that the .1g concentration was most efficient, even more so than the baseline when it came to an increase in development time. Some possible limitations that could have come along with these results could have been our dosages not being perfect or even that we were dealing with different individual embryos which could have had different reactions we didn’t notice.



Discussion

Because the data set is simply not large enough to do statistical tests, it is difficult to properly use the word ‘significant’ when describing the data. One observation between the different wells was that, in the first two days many of the fish exposed to some or little creatine did not seem to be affected. With more experimentation we could find if creatine has a significant impact on embryo development. Our data does not support our hypothesis as we predicted that more creatine monohydrate would cause a greater increase in development while there was a peak amount that the fish could handle and flourish under. This data connects to human consumption of creatine which goes off of body weight.

References

-Twycross-Lewis, R. "The effects of creatine supplementation on thermoregulation and physical (cognitive) performance: a review and future prospects." *EBSCOhost*. Springer, Aug. 2016. Web. Nov. 2016.
-Brosnan, Margaret and John. "The role of dietary creatine." *EBSCOhost*. Springer, Aug. 2016. Web. Nov. 2016.