Impact Factor (SJIF): 6.092 E-ISSN: 2583-1615

International Journal of Research in Academic World

Received: 06/August/2023 IJRAW: 2023; 2(9):05-08 Accepted: 03/September/2023

Creatine Monohydrate Supplementation Increase Heat Resistance in *Drosophila Melanogaster*

[1]Sithembiso Sebastian Mamba and [*2]Mysore Siddaiah Krishna

[1, *2]Department of Zoology, University of Mysore, Manasagangotri, Mysore, Karnataka, India.

Abstract

Environmental factors are instable particularly in the present days hence the survival and continuity of organisms is immensely affected. Extreme temperatures, dehydration, and food deprivation as well as the type of nutrition may have diverse effects on different age groups of males and females across species. In the present study, the effect of creatine monohydrate on heat resistance in *Drosophila melanogaster* was investigated. The results demonstrated that flies maintained on creatine monohydrate supplemented media were more resistant to heat compared to flies maintained on control media and female flies also showed significantly higher heat resistance compared to male flies in both mated and unmated flies. Heat resistance increased with increased creatine monohydrate supplementation. It was also discovered that unmated flies were more resistant to heat than their mated counterparts. Creatine monohydrate enhance heat resistance in *Drosophila melanogaster*.

Keywords: Creatine monohydrate, *Drosophila Melanogaster*, heat resistance

Introduction

A crucial component of habitat quality is the likelihood that an organism may experience excessive heat stress (Huey, 1991). Resistance to thermal extremes may be closely correlated with fitness for organisms living in microenvironments with changing temperatures. Regarding any evolutionary response, Krebs and Loeschcke (1994) [16] connected resistance with colonization of new settings or persistence through a state of change. Along with increases in mean temperature, organisms are subjected to longer, more intense, and more frequent episodes of heat stress (IPCC, 2014) [12]. According to numerous studies (e.g., Colinet *et al*., 2015; Estay *et al*., 2014; Ma *et al*., 2021; Rodrigues & Beldade, 2020; Sheldon & Dillon, 2016) [4, 9, 20, 22, 25], this temperature variability will be a significant factor in how susceptible organisms are to global warming. Under conditions of heat stress, high temperatures can have an impact on the reproduction, abundance, and dispersion of species in contemporaneous natural ecosystems (Franks and Hoffmann, 2012; Hoffmann and Sgro, 2011) [13, 11]. (Kellermann *et al*., 2009; Kellermann *et al*., 2012; Kingsolver *et al*., 2011; van Heerwaarden *et al.,* 2016;) [14, 13, 27] are just a few examples of the studies that show how important it is for individuals to be able to respond to adverse environmental conditions like thermal stress for adaptation to climate changes, including global warming.

The model organism *Drosophila melanogaster* has been extensively employed in the scientific sciences. *Drosophila* is a popular choice for life span studies because of its 60-80 day lifespan. Additionally, 60% of the fruit fly genes have mammalian orthologs. As a result, metabolic and signaling pathways are much conserved (Staats *et al.*, 2018) [26]. *Drosophila* maintenance and reproduction are relatively inexpensive and don't call for expensive equipment. Comparatively to studies using laboratory rodents like rats and mice, experimental *Drosophila* research has fewer ethical concerns. The use of *Drosophila* as a model organism in food and nutrition research is growing. Feed intake, body composition, locomotor activity, intestinal barrier function, microbiota, cognition, fertility, aging, and life span can be systematically determined in *Drosophila* in response to dietary factors (Staats *et al.*, 2018) [26]. Furthermore, diet-induced pathophysiological mechanisms including inflammation and stress responses may be evaluated in the fly under defined experimental conditions.

Creatine is a naturally occurring substance that is created in the liver, pancreas, and kidneys as well as ingested in the diet, primarily in the form of meat and fish (3-5 g/kg raw meat). Creatine was discovered in 1832 by Chevreul, and it was recognized nearly a century later that creatine is essential for the generation of energy during muscular contraction. Adenosine diphosphate (ADP) and the enzyme creatine kinase react with creatine, phosphocreatine (PCr), and creatine in skeletal muscle to resynthesize adenosine triphosphate (ATP) in humans (Sahlin, 2014) [24]. Creatine is an example of an amino acid, which are the building blocks of proteins. Among other things, proteins support the development of bones and muscles and aid in tissue repair.

*Corresponding Author: Mysore Siddaiah Krishna

Your skeletal muscles can flex (contract) with the aid of creatine, a natural source of energy. In particular while you're exercising, it aids in generating a consistent flow of energy in your muscles so they can continue to function. Up to 80% of the ATP produced during brief, intense exertion, such sprinting, is created via the creatine kinase process (Rawson, 2018) [21].

The quantity and quality of nutrients that organisms ingest have a significant impact on their ability to withstand environmental stress, life-history traits, and reproductive success. The ability of an organism to resist stress can be affected by a variety of factors through behavioral and physiological changes. Additionally, it was shown that an organism's exposure to climatic variations might result in physiological modifications including the hardening process, coma, the synthesis of metabolites, and the development of a tolerance for temperature extremes (Kostal *et al*., 2007) [15]. The present study has been conducted to investigate to effect of creatine monohydrate on heat resistance heat resistance in *Drosophila melanogaster*.

Materials and Methods

Establishment Stocks

The *Drosophila* Stock Centre at the Department of Zoology at the University of Mysore in Karnataka, India, provided the Oregon-K strain of *D. melanogaster* flies. For two generations, these flies were bred to create the experimental stock. On culture bottles containing wheat cream agar media, the flies were kept under laboratory conditions. The temperature and relative humidity in the lab where the culture bottles were stored were around 22^0C $\pm 1^0$C degrees Celsius and 70%, respectively.

Wheat cream agar-agar was used to cultivate the flies that were used as control. The wheat cream Agar-agar media was supplemented with creatine monohydrate at varied percentages (2.5%, 5%, and 10%) for the experimental diets. The Synergy Supplement Store in Mysore, Karnataka, India is where the creatine monohydrate supplement was purchased. Separately, twenty flies were transferred to wheat cream agar media and creatine monohydrate supplemented media. These flies were kept in lab conditions as previously indicated. For this experiment, flies collected from the culture bottles were utilized.

Effect of Creatine Monohydrate on Environmental Fitness (Heat Resistance)

Four to five days old flies and mated flies raised from control media and CrM supplemented media (2.5%, 5% and 10%) were kept in an incubator at a temperature of 37^0C (degrees Celsius) until each one of them died. The flies were separated into mated male, mated females, unmated males and unmated females. The time on which they are put in the incubator was noted and there were observed every five (5) minutes. Dead flies were counted and its survival time noted. Five vials (replicates) each containing 20 flies per treatment was placed evenly spaced in incubators. On the aforementioned data, two way ANOVA followed by Tukey's Post Hoc Test was undertaken.

Results and Discussion

Mated Flies: The findings demonstrated that mated *Drosophila melanogaster* females maintained on media supplemented with 10% CrM are more heat tolerant than females maintained on media supplied with 2.5% and 5% CrM. Female flies kept on control media were much less heat resistant than female flies given 10% CrM supplemented media. The study found no discernible difference between the heat resistance of mated *Drosophila melanogaster* males kept on diets supplemented with 2.5% CrM and 5% CrM. However, compared to the control and male flies maintained on media supplemented with 10% CrM, they both had significantly different heat resistance. In comparison to the male flies maintained on the same CrM supplemented diets, males maintained on control diets had significantly lower mean heat resistance. Figure 1 shows the mean heat resistance of both mated male and mated female flies and the mean standard error bars.

When comparing the mean heat resistance between mated male flies and mated female flies, the results showed that mated female flies are significantly more resistance to heat than the mated male flies on 10% CrM supplemented media, 5% CrM supplemented media and the control media. There was no significant difference in the mean heat resistance of mated male flies and mated female flies maintained on 2.5% CrM supplemented media. The results also showed that increasing dosage of CrM supplement in the diet enhanced or improved the mean heat resistance of mated male and female flies. The bar chart on figure below shows the mean heat resistance of mated male and female *Drosophila melanogaster* for each treatment with its standard errors.

When subjected to ecologically significant temperature changes, adults of *D. melanogaster*, like many other insects, can quickly enhance their thermal tolerance (Chenet *et al*., 1987). The obtained results indicates that creatine monohydrate enhance heat tolerance in *D. melanogaster* since all the flies were subjected to the same conditions except creatine supplementation. The female flies were observed to be more resistant to heat compared to their male counterparts. According to Carvalho *et al*. (2006) [2] and Lee *et al*. (2013) [19], mated females ingested more food and accrued more lipids than male. Balogh *et al*., (2013) [1] stated that membranes are the macromolecular structures that are most susceptible to temperature changes because of their distinct molecular arrangement. Furthermore, environmental (stress) variables trigger the activation of lipid metabolic enzymes and their downstream targets, which together form a complex lipid signaling network with a number of focal areas for interactions and cross-talk via their lipid mediators.

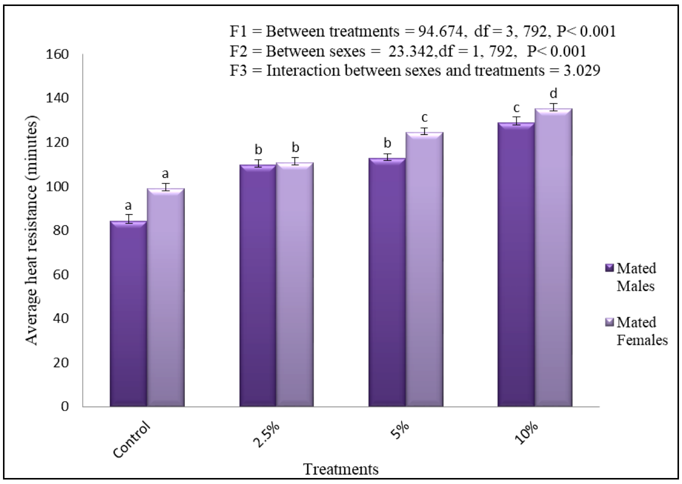

Fig 1: Effect of creatine monohydrate on heat resistance of mated *Drosophila melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test.

Unmated Flies

The results showed that the mean heat resistance of unmated male flies was significant for the experimental diets (treatments) including the control. Unmated male flies maintained on 10% CrM supplemented showed the highest mean heat resistance compared to other treatments. Mean heat resistance was observed to decrease with reduced dosage of CrM supplement in the diet in unmated males. The study showed that supplementing *Drosophila melanogaster* diet with CrM supplement increases its heat resistance. The mean heat resistance of Unmated female flies increased with increased dosage of CrM supplement and was significantly different in the all the treatments with unmated female flies maintained on 10% CrM supplement having higher mean heat resistance. Fig. 2 shows the mean heat resistance of unmated *Drosophila melanogaster*. The mean heat resistance of unmated female flies was observed to be significantly higher when compared to the mean heat resistance of unmated male flies across all the treatments. Unmated flies maintained on control media had the least resistance to heat in both sexes and the resistance to heat increased with increase in creatine monohydrate supplementation.

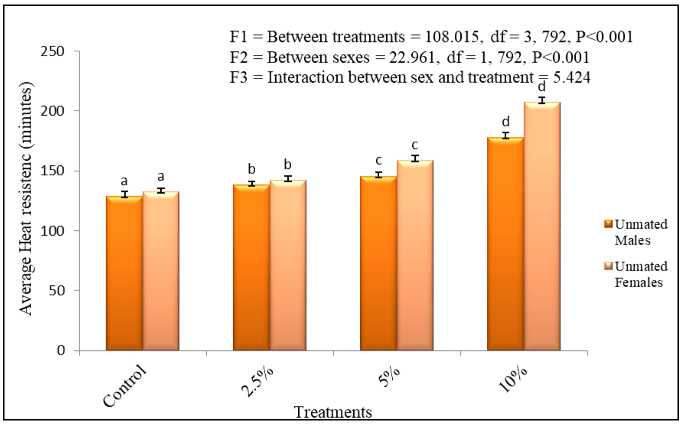

Fig 2: Effect of creatine monohydrate on heat resistance of unmated *Drosophila melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test.

Heat Resistance between Male Flies (Mated and Unmated)

The average heat resistance of mated male and unmated male *Drosophila melanogaster* is shown in Figure 3. Across all of the treatments, it was shown that the mean heat resistance of unmated male flies was considerably higher than the mean heat resistance of mated male flies. The least resistant to heat in both sexes were mated male flies kept on control media, and the resistance to heat was observed to rose with increasing supplementation of creatine monohydrate.

The obtained result meant that mated male flies less lipid reserves compared to unmated male flies since they uses energy more including during mating. In several sets of *Drosophila* lines selected for changes in life history traits, Chippindale *et al.* [6] examined the quantities of lipid and heat resistance. They found that lipid content accounts for virtually all of the variation in resistance. After analyzing all the data, they found a nearly one-to-one correlation between cholesterol levels and resistance. Heat resistance and relative fat content were found to be positively associated by Zwaan *et al.* [31] who also looked into the relationship between the two variables.

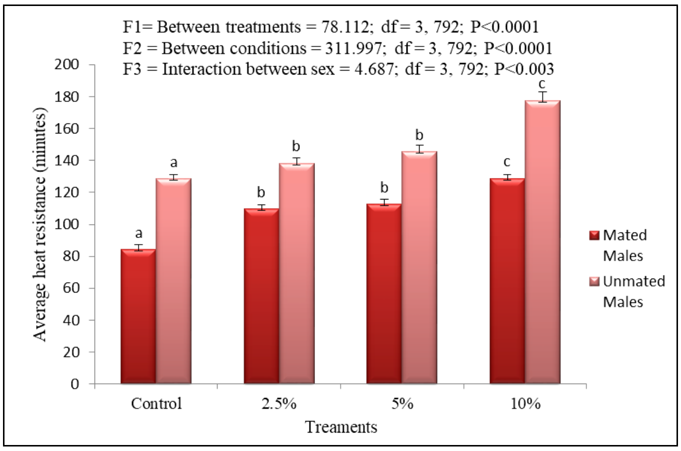

Fig 3: Effect of creatine monohydrate on heat resistance of mated and unmated male *Drosophila melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test.

Heat Resistance between Female Flies (Mated and Unmated)

Figure 4 displays the average heat resistance of mated and unmated female *Drosophila melanogaster*. It was discovered that the mean heat resistance of mated female flies was significantly higher than the mean heat resistance of unmated female flies across all treatments. The unmated female flies fed on control media were the least heat-resistant in both sexes, and it was shown that heat resistance increased with increasing dosage of creatine monohydrate.

Rush *et al.* [22] claim that increase in food consumption and its associated increase in lipid storage are the main causes of the rise in heat resistance in female *D. melanogaster*. Unmated female flies had high energy reserves in the form of lipid compared to the mated female flies. The observed difference can be attributed to nutrient utilization whereby mated females utilizes its energy for egg production and other post-mating energy demands while unmated female has no energy expenditure on post-mating energy usage.

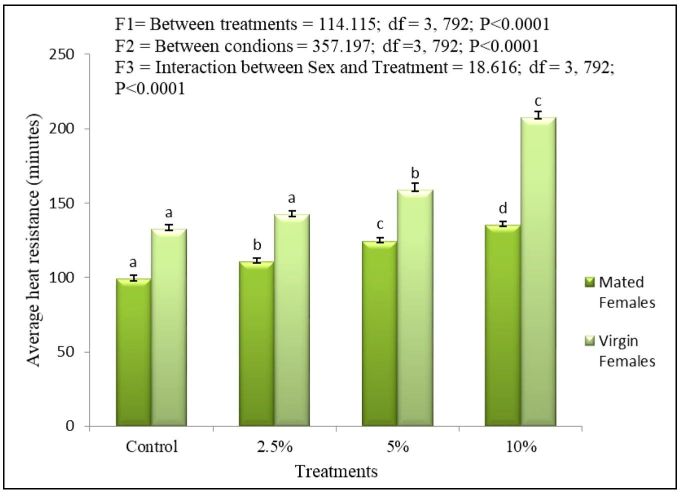

Fig 4: Effect of creatine monohydrate on heat resistance of mated and unmated female *Drosophila melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test.

Conclusion

According to the *D. melanogaster* study, using creatine monohydrate supplements considerably improves heat resistance. In *D. melanogaster*, female flies are more heat-resistant than male flies.

Acknowledgements

The authors are indebted to the Chairperson of the Department of Studies in Zoology, *Drosophila* Stock Center, University of Mysore, Manasagangotri campus, Mysuru, for providing all the equipment required for the successful completion of the study. Sincere gratitude to each and every professor and lecturer in the department of zoology.

References

1. Balogh G, Péter M, Glatz A, Gombos I, Török Z, Horváth I, Harwood JL, Vígh L. Key role of lipids in heat stress management. FEBS Lett. 2013; 587(13):1970-80.
2. Carvalho AP, Meireles LA, Malcata FX. Microalgal reactors: A review of enclosed system designs and performances. Biotechnology progress. 2006; 22(6):1490-1506.
3. Chippindale AK, Chu TJF, Rose MR. Complex trade-offs and the evolution of starvation resistance in Drosophila melanogaster. Evolution. 1996; 50:753-766.
4. Colinet H, Sinclair BJ, Vernon P, Renault D. Insects in fluctuating thermal environments. Annual Review of Entomology. 2015; 60:123-140.
5. Cooper R, Naclerio F, Allgrove J, Jimenez A. Creatine supplementation with specific view to exercise/sports performance: an update. *J Int Soc Sports Nutr*. 2012; 9(1):33.
6. Cooper R, Naclerio F, Allgrove J, Jimenez A. Creatine supplementation with specific view to exercise/sports performance: an update. *Journal of the International Society of Sports Nutrition*. 2012; 9(1):33.
7. Costantini D. Oxidative stress and hormesis in evolutionary ecology and physiology. Springer, 2014.
8. Crawley MJ. The R book. John Wiley & Sons Ltd, 2007.
9. Estay SA, Lima M, Bozinovic F. The role of temperature variability on insect performance and population dynamics in a warming world. Oikos. 2014; 123:131-140.
10. Franks SJ, Hoffmann AA. Genetics of climate change adaptation. Annu Rev Genet. 2012; 46:185-208.
11. Hoffmann AA, Sgrò CM. Climate change and evolutionary adaptation. Nature. 2011; 470(7335):479-85.
12. IPCC. Climate change 2014: Synthesis report. In E. Core Writing Team, Pachauri RK, Meyer LA (Ed.), Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, 2014, 151.
13. Kellermann V, Overgaard J, Hoffmann AA, Fløjgaard C, Svenning JC, Loeschcke V. Upper thermal limits of Drosophila are linked to species distributions and strongly constrained phylogenetically. Proc Natl Acad Sci U S A. 2012; 109(40):16228-33.
14. Kellermann V, van Heerwaarden B, Sgrò CM, Hoffmann AA. Fundamental evolutionary limits in ecological traits drive Drosophila species distributions. Science. 2009; 325(5945):1244-6.
15. Koštál D. Renault, A. Mehrabianová, J. Bastl, Insect cold tolerance and repair of chill-injury at fluctuating thermal regimes: Role of ion homeostasis, Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 2007; 147(1):231-238,
16. Krebs RA, Loeschcke V. "Costs and Benefits of Activation of the Heat-Shock Response in Drosophila Melanogaster." *Functional Ecology*. 1994; 8(6):730-37. *JSTOR*.
17. Kristensen TN, Loeschcke V, Tan Q, Pertoldi C, Mengel-From J. Sex and age specific reduction in stress resistance and mitochondrial DNA copy number in Drosophila melanogaster. Sci Rep. 2019; 9(1):12305.
18. Vigh L, Maresca B, Harwood JL. Does the membrane's physical state control the expression of heat shock and other genes? Trends Biochem. Sci. 1998; 23:369-374.
19. Lee JW, Kil DY, Keever BD, Killefer J, McKeith FK, Sulabo RC, Stein HH. Carcass fat quality of pigs is not improved by adding corn germ, beef tallow, palm kernel oil, or glycerol to finishing diets containing distillers dried grains with solubles. *J Anim. Sci.* 2013; 91(5):2426-2437.
20. Ma CS, Ma G, Pincebourde S. Survive a warming climate: Insect responses to extreme high temperatures. Annual Review of Entomology. 2021; 66:163-184.
21. Rawson ES, Miles MP, Larson-Meyer DE. Dietary Supplements for Health, Adaptation, and Recovery in Athletes. *Int J Sport Nutr Exerc Metab*. 2018; 28(2):188-199.
22. Rodrigues YK, Beldade P. Thermal plasticity in insects' response to climate change and to multifactorial environments. Frontiers in Ecology and Evolution. 2020; 8:271.
23. Rush B, Sandver S, Bruer J, Roche R, Wells M, Giebultowicz J. Mating increases starvation resistance and decreases oxidative stress resistance in Drosophila melanogaster females. Aging cell. 2007; 6(5):723-726.
24. Sahlin K. Muscle energetics during explosive activities and potential effects of nutrition and training. Sports Med. 2014; 2(2):S167-73.
25. Sheldon KS, Dillon ME. Beyond the mean: Biological impacts of cryptic temperature change. Integrative and Comparative Biology. 2016; 56:110-119.
26. Staats S, Lüersen K, Wagner AE, Rimbach G. Drosophila melanogaster as a Versatile Model Organism in Food and Nutrition Research. *Journal of Agricultural and Food Chemistry*. 2018; (15):3737-3753.
27. Van Heerwaarden B, Kellermann V, Sgro CM. Limited scope for plasticity to increase upper thermal limits. *Func Ecol*. 2016; (12):1947-56.
28. Zwaan B, Bijlsma R, Hoekstra R. On the developmental theory of ageing. I. Starvation resistance and longevity in Drosophila melanogaster in relation to pre-adult breeding conditions. Heredity. 1991; 66:29-39.