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Research Article

CREATINE MONOHYDRATE SUPPLEMENTATION DECREASES STARVATION RESISTANCE OF *DROSOPHILA MELANOGASTER*

Sithembiso Sebastian Mamba and Mysore Siddaiah Krishna*

Drosophila Stock Centre, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore, 560006, Karnataka, India

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ABSTRACT

In the current study, flies from control and creatine monohydrate-treated medium were utilized to investigate the effect of creatine monohydrate in starvation resistance of *Drosophila melanogaster*. It was discovered that flies fed with control diet had significantly higher starvation resistance compared to flies maintained on creatine monohydrate treated media and starvation resistance decreased with increase creatine monohydrate concentration. It was also observed that female *Drosophila melanogaster* had significantly higher starvation resistance compared to both their mated and unmated male counterparts. It was further discovered that mated female were more resistant to starvation than unmated female flies whilst mated male flies were less resistant to starvation compared to unmated male flies. With observation made from the study, it was concluded that creatine monohydrate supplementation reduce starvation resistance in *Drosophila melanogaster* suggesting its negative effects on starvation resistance

INTRODUCTION

Food scarcity is the most prevalent environmental difficulty that animals experience, and animals' ability to withstand protracted periods of famine is tied to their food and nutritional state. The ability of animals to withstand prolonged periods of food deprivation is known as 'starvation resistance' (SR), and it is a phenotypic trait of great organismal, ecological, and evolutionary significance because starvation is the most prevalent environmental stress faced by animals living in environments where food availability fluctuates and is unpredictable [16]. Starvation induces stress in animals and is one of the environmental conditions that reduces an organism's fitness, hence it plays an important part in natural selection. It is possible to characterize biological stress in terms of evolution [13].

Stress is widely defined by Sibly and Calow [24] as an environmental situation that, when first applied, reduces Darwinian fitness, and by Koehn and Bayne [14] as any environmental change that affects an organism's fitness. The degree to which genetic variations in stress tolerance lead to adaptive change depends on how frequently an organism is exposed to its environment and the physiological costs involved [13]. Environmental stress is defined as the lack of acceptable or sufficient food supplies, which deprives a population of normal nutrients [30]. It has been stated that stress related to few resources has an impact on the populations of most species. Selection probably influences resistance traits either directly or indirectly since stress resistance traits in Drosophila frequently differ across latitudinal clines [26]. Many species' individuals must endure times of famine or exposure to unsatisfactory nutrition. In areas where food is likely to be less plentiful or momentarily less consistent, positive selection for resistance to famine stress is anticipated. As is frequently observed when insects are restricted to food low in protein relative to carbohydrate [17] compensatory feeding for the limiting nutrients results in the over-ingestion of other nutrients when faced with nutritionally imbalanced diets. This may increase lipid storage and reduce fitness [25, 29].

The use of Drosophila as a model organism in food and nutrition research is growing. Under specific experimental conditions, flies may be used to assess the pathophysiological mechanisms of diet, such as inflammation and stress responses [27]. The fruit fly, *Drosophila melanogaster* is a potent model organism for examining the molecular underpinnings of starvation resistance [20].

Creatine monohydrate is a dietary supplement that increases muscle performance in short-duration, high-intensity resistance exercises, which rely on the phosphocreatine shuttle for

Corresponding author:* **Mysore Siddaiah Krishna
Drosophila Stock Centre, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore, 560006, Karnataka, India

adenosine triphosphate [4, 10]. Creatine is produced by the combination of three amino acids-glycine, arginine, and methionine and three enzymes L-arginine:glycine amidinotransferase, guanidinoacetate methyltransferase, and methionine adenosyltransferase [7]. It is frequently claimed that taking creatine supplements and engaging in vigorous resistance training improves physical performance, lean body mass, and muscular morphology [28] hence the sudden surge in the utilization of creatine monohydrate supplement in the present era. Even though food is not always a reliable supply, it is yet unknown how a creatine monohydrate supplement may affect starvation. This investigation looks into how starving of *Drosophila melanogaster* is affected by creatine monohydrate supplementation.

MATERIALS AND METHODS

Establishment stocks

Oregon-K strain of *D. melanogaster* flies were obtained from the Drosophila Stock Centre at the Department of Zoology in the University of Mysore, Karnataka, India. These flies were bred for two generations to establish the experimental stock. The flies were kept on culture bottles containing wheat cream agar media. The culture bottles were kept in the laboratory condition with temperature around 22^0c ± 1^0c (degrees Celsius) and a relative humidity of 70%.

Wheat cream Agar-agar media was utilized to culture control flies. The experimental diets were based on CrM which was supplemented at different percentages (2.5%, 5% and 10%) to the starvation cream Agar-agar media. The CrM supplement was procured from Synergy Supplement Store, Mysore, Karnataka, India. Twenty flies were transferred separately to culture bottles of wheat cream agar media and creatine monohydrate treated media. The treated flies were maintained in laboratory condition as mentioned above. The flies obtained from the culture bottles were used for the present experiment.

Effect of creatine monohydrate on environmental fitness (starvation)

Starvation resistance was measured using mated flies and 4–5 days old unmated flies of control and CrM treated media. Ten (10) flies of each sex were measured for each diet. Four replicates were carried out. To measure starvation resistance, flies from each control and the respective CrM supplemented media were transferred separately to a new vial containing 1% agar media and plugged with cotton in order to prevent desiccation. Time was noted down during transferring of the flies. Starvation resistance was observed for 2 hour intervals until death of each fly. Two way ANOVA followed by Tukey's Post Hoc Test was carried out on above data.

RESULTS

Mated flies

Mated female flies were more resistant to starvation than mated male flies. The mean starvation resistance recorded in hours is represented in **Fig 1.** Mated flies maintained on 10% creatine monohydrate supplemented media in both sexes (male and female) had the least resistance to starvation compared to all the creatine monohydrate supplemented media and the control. Mated female control flies showed higher mean starvation resistance compared flies fed creatine monohydrate supplemented media.

The above data subjected to two way ANOVA followed by Tukey's post hoc test showed significant variations in time taken by mated male and female flies from respective creatine monohydrate supplemented media and control to survive without food. Interaction between treatments (creatine monohydrate supplemented media and control) and sexes also gave significant variations (Fig. 1).

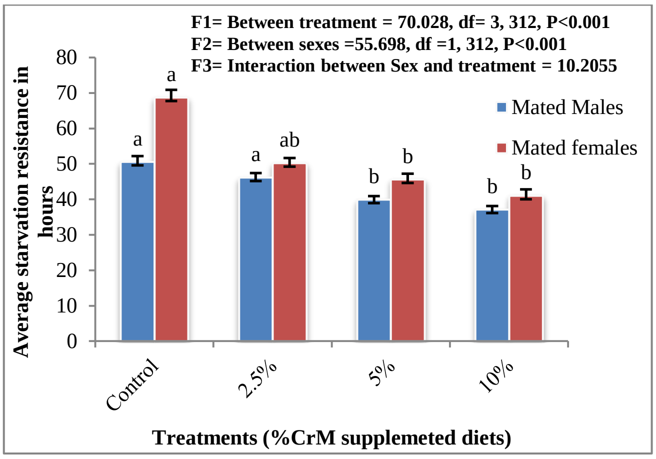

Figure 1 Effect of creatine monohydrate on starvation resistance mated male and female of *Drosophila melanogaster*.

Different letters on the bar graph indicates significant variation at 0.05 levels by Tukey's Post Hoc test.

Unmated flies

Unmated female flies were less susceptible to hunger compared to unmated male flies. **Fig. 2** shows the mean starvation resistance measured in hours. In comparison to all the creatine monohydrate added media and the control, unmated flies maintained on 10% creatine monohydrate supplemented media in both sexes (male and female) showed the lowest resistance to famine. In comparison to flies fed media supplemented with creatine monohydrate, mated female control flies demonstrated higher mean hunger resistance.

Two-way ANOVA followed by Tukey's post hoc test results revealed significant differences in starvation resistance between unmated male and female flies from control and medium supplemented with creatine monohydrate, respectively. Additionally, there was a significant difference between the sexes and treatments (creatine monohydrate supplemented medium and control) (Fig.2).

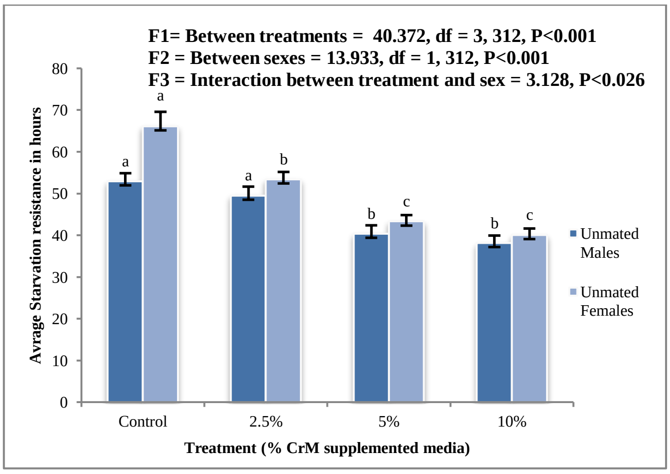

Figure 2 Effect of creatine monohydrate on starvation resistance unmated male and unmated female of *Drosophila melanogaster*.

Different letters on the bar graph indicates significant variations at 0.05 level by Tukey's Post Hoc test.

Mated and unmated females

Fig. 3 show the mean starvation resistance of mated female flies and unmated females flies. Mated females showed higher resistance to starvation compared to unmated females in all the respective creatine monohydrate supplemented media and the control. Both mated female and unmated female flies maintained on 10% creatine monohydrate supplemented media had the least resistance to hunger compared to all the other treatments and the control.

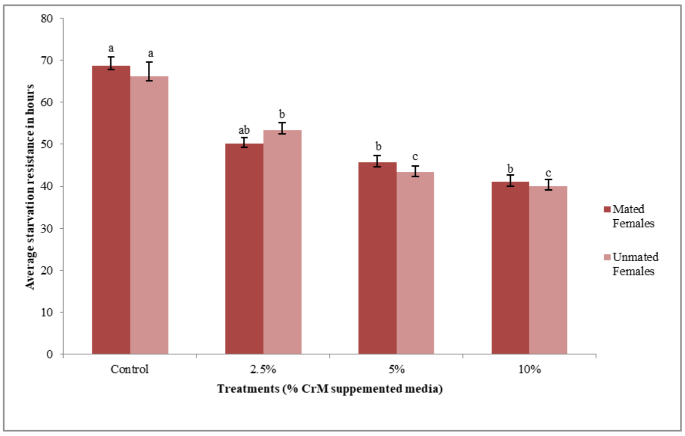

Figure 3 Effect of creatine monohydrate on starvation resistance of female *Drosophila melanogaster* (mated female and unmated female).

Different letters on the bar graph indicates significant variation at 0.05 level by Tukey's Post Hoc test.

Mated and unmated males

The average starvation resistance of mated and unmated male flies is shown in **Fig. 4.** In all of the corresponding creatine monohydrate supplemented media as well as the control, unmated male flies demonstrated greater hunger resistance than mated male flies. In comparison to all other treatments and the control, unmated male flies and mated male flies fly maintenance on 10% creatine monohydrate supplemented media showed the lowest resistance to hunger. Control flies showed the highest resistance to starvation in both unmated male flies and mated male flies.

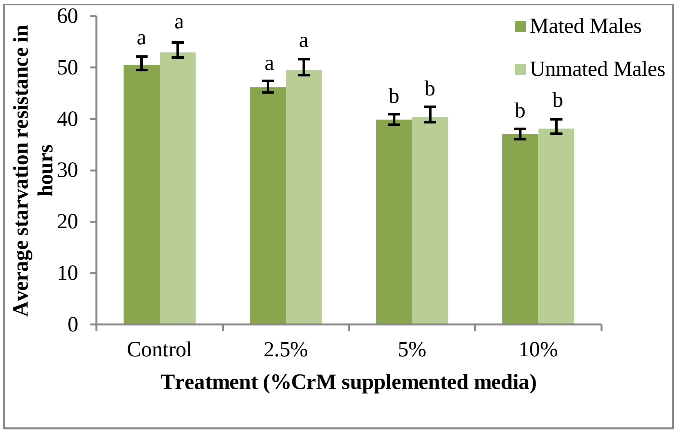

Figure 4 Effect of creatine monohydrate on starvation resistance of male *Drosophila melanogaster* (mated male and unmated male).

Different letters on the bar graph indicates significant variation at 0.05 level by Tukey's Post Hoc test.

DISCUSSION

The food is required for all the animals for the growth, development, physiology, health, starvation resistance and survival of an organism. However, the availability of food and their nutrients vary from different diets. Therefore the presence study has been undertaken to understand the effect of creatine monohydrate on starvation resistance. Creatine monohydrate is a dietary supplement that increases muscle performance in short-duration, high-intensity resistance exercises, which rely on the phosphocreatine shuttle for adenosine triphosphate [10]. Our results demonstrated that flies maintained on control media were more resistant to starvation compared to flies from treated media and starvation resistance decreased with increase media supplementation using creatine monohydrate. This suggest that flies maintained on control media accumulated more fats or lipids during their development than the flies maintained on CrM treated media and fat accumulation decrease with increase in CrM supplementation. This results were consistent with the findings of Deminice *et al.* [8], who while working on rats have also found that creatine supplementation prevented the hepatic fat accumulation that occurs upon feeding a high-fat diet to rats for 3 wk. This means that the more CrM present in the diet, the less fat the flies accumulate and, thus, the less resistant they are to starvation. There is strong evidence that adults' enhanced tolerance to hunger is caused by an increase in their lipid content. According to Chippindale *et al.*, [6], who measured the amounts of lipid and starvation resistance in various sets of *Drosophila* lines chosen for starvation or changes in life history features, lipid content is responsible for nearly all of the variance in starvation resistance. When they took all lines into account, they discovered a link between fasting and lipid levels that was almost one. In a similar study, Zwaan *et al.* [31], investigated the relation between starvation resistance and relative fat content and discovered that starvation resistance and relative fat content were positively correlated. Further Lee and Jang [15] discovered that *Drosophila* were better able to resist starvation by sequestering more lipid reserves when fed on diets containing higher proportion of carbohydrate to protein. Further in our study, it was noticed that starvation resistance decreases with increased creatine monohydrate concentration in the diet.

In the present study we also found that females had significantly greater starvation resistance compared to males both when the flies are mated (Figures 1) and unmated (Figure 2). This is because in *Drosophila melanogaster*, female flies were bigger than males because females might have accumulated more lipids in relation to their body weight as suggested by Hoffmann and Harshman [12] that body weight may be an indicator of the total amount of energy-storing chemicals that an organism is carrying. Further Lee and Jang [14] verified that when body lipid reserves were entirely depleted in flies, starvation-induced death took place [18].

According to our data, which were consistent with earlier studies [3, 21], mated females were more resistant to starvation than mated males (Figure 1). The main cause of the rise in hunger resistance in female *D. melanogaster* is the post-mating increase in food consumption and its associated increase in lipid [22]. A recent study found that absorption (autophagic reabsorption of oocytes) did not increase SR in female *Drosophila*, despite the possibility that female flies could utilize nutrients reabsorbed from eggs to survive famine [2]. Starvation resistance was inversely related CrM supplementation (SR decrease with increase in CrM supplementation). CrM do not have any fat or lipid forming compounds and studies have shown that lead to increase in lean muscle size thus leading to more body mass other than body fat or muscle fat. Therefore, flies from treated media had less lipid hence less resistant to starvation.

In the present study, we also studied the effect of creatine monohydrate on starvation resistance of unmated and mated *Drosophila melanogaster*. We found that unmated male flies were significantly higher resistant to starvation compared to mated male flies (Figure 4) and in contrast to this mated female had significantly higher resistance to starvation compared to unmated female flies (Figure 3). It was also observed that starvation resistance decreased with increasing creatine monohydrate supplementation in the diet in both sexes. It has been demonstrated that mating has significant benefits on starvation resistance in *Drosophila* species [9, 22, 23]. In female *D. melanogaster*, mating is known to stimulate egg production, suppress sexual receptivity, and increase food intake. This effect is primarily mediated by male seminal fluid peptides transferred to females [5, 11, 17]. According to Rush *et al*. [22], the main cause of the rise in hunger resistance in female *D. melanogaster* is the post-mating increase in food consumption and its associated increase in lipid storage. In more recent research, it has also been shown that female *D. melanogaster'*s midguts can grow significantly as a result of mating, allowing mated females to meet their increased energy needs for egg production by maximizing their post-ingestive nutrient utilization [18]. Males under starvation stress metabolized solely body lipids as a source of energy, whereas females under starvation stress stored larger (1.3-fold) dry-mass-specific levels of body lipids and glycogen contents and used both of these energy resources [1]. From the present study it was concluded that creatine monohydrate treated diet reduces starvation resistance in *Drosophila melanogaster.*

CONCLUSION

The study in *D. melanogaster* revealed that creatine monohydrate supplementation significantly reduce starvation resistance suggesting that creatine monohydrate affects survival under food stress conditions in *D. melanogaster*.

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