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Creatine Monohydrate Decrease Longevity in *Drosophila Melanogaster*

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Abstract

Creatine monohydrate supplement usage has surged nowadays. It is however unknown whether it has benefit or detrimental effect on organisms fitness other than the well-researched ergogenic effect. The current study has been undertaken to find the effect of creatine monohydrate on longevity in *Drosophila melanogaster*. In the current study, *Drosophila melanogaster* longevity was investigated using flies fed control media and creatine monohydrate supplement treated media at different concentrations (2.5%, 5% and 10%). It was observed that control flies lived noticeably longer than flies maintained on creatine monohydrate supplemented media in both sexes. However, females showed longer survival time compared to male flies. The study further revealed that longevity decrease with increase in creatine monohydrate supplementation. The results demonstrated that creatine monohydrate reduce longevity in *D. melanogaster*.

Keywords: Longevity, Creatine monohydrate, and *Drosophila melanogaster*

Introduction

A vital extrinsic component that can impact an organism's growth, development, and survival is its nutrition. Diet is a significant extrinsic factor that can influence an organism's growth, development, and survival (Sisodia and Singh, 2012) [19]. It was demonstrated that dietary restrictions without malnutrition affect lifespan and reproductive output in a variety of organisms, from nematode worms to mammals (Piper *et al*., 2011) [17] studies have emphasized that it was diet that had a significant impact on life span and reproductive output (Piper *et al*., 2011; Taylor and Fanson, 2012) [17, 6]. It was also demonstrated that the type of diet an organism consumes and how it affects its life history traits can be divided into two categories: quantity and quality. It depends on food availability and quality, which depends on food's nutritional makeup (Sisodia and Singh, 2012) [19]. In many species, there are disparities between the average lifespan of the sexes, and females often outlive males (Lints *et al*. 1983; Austad and Fischer 2016) [3]. The reported variations in lifespan between the sexes are frequently inferred from wild populations and attributed to risk-taking behavior, feeding habits, and sexual competition, which may have little to do with sex differences in intrinsic aging rates (Austad and Fischer 2016) [3]. The sexual selection theory (Trivers 1972) [21] postulates that lifelong investment and reproductive effort trade-off, resulting in sex differences in ROS generation and antioxidant defenses. However, it is still unclear how oxidative stress affects sexual selection. It can be helpful to understand sex-specific aging by comparing the lifespans of the sexes within a species (Austad and Fischer 2016) [3].

In brief, high-intensity resistance activities that rely on the phosphocreatine shuttle for adenosine triphosphate, a nutritional supplement called creatine monohydrate (CrM) improves muscle function (Hall, 2013) [9]. It is a well-liked ergogenic aid among athletes. According to Cooper *et al*. (2012) [4], three enzymes-L-arginine:glycine amidinotransferase, guanidinoacetate methyltransferase, and methionine adenosyltransferase-along with three amino acids-glycine, arginine, and methionine-combine to form creatine. Creatine supplementation plus intense resistance exercise are said to enhance physical performance, lean body mass, and muscle morphology (Volker *et al*., 1999) [22]. Additionally, a growing body of evidence (Jagim and Kerksick, 2021) [24] supports the therapeutic benefits of creatine supplementation for a variety of clinical purposes in both adults and children. Since it is widely believed that combining rigorous resistance exercise with creatine monohydrate supplements improves physical performance, lean body composition, and muscle morphology, the use of these supplements has abruptly expanded in the modern era (Volker *et al*., 1999) [22]. Despite the extensive evidence showing that creatine monohydrate supplements are safe and effective in physical fitness, their holistic effect on organisms' fitness particularly longevity is mysterious. The current study has been undertaken to determine the effect on creatine monohydrate on longevity on *Drosophila melanogaster.*

Materials and Methods

Stock Establishment

*Drosophila*stock center, Department of Zoology, University of Mysore, Manasagangothri, Mysuru provided the Oregon K

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strain of *Drosophila melanogaster* that was utilized in the study to create experimental stock. The stock was cultivated in bottles containing wheat cream agar media, which were made by boiling 100g of wheat cream powder, 100g of jaggery, and 10g of agar in 1000ml of distilled water and added 7.5ml of propionic acid. Flies were kept in a lab environment with 70% humidity, 12-hour cycles of darkness and light, and a constant temperature of 22°C plus or minus 1°C. The investigations were carried out using the flies.

Experimental Stock

Wheat cream Agar-agar was used as the culture medium for control flies. To form the foundation of the experimental diet, different concentrations of Creatine monohydrate (2.5%, 5%, and 10%) were added to the wheat cream Agar-agar media. The Synergy Supplement Store in Mysore, Karnataka, India supplied the Creatine monohydrate supplement used in the study. Twenty flies were transferred, ten males and ten females, individually to culture bottles containing wheat cream agar media and the medium treated with creatine monohydrate. As previously mentioned, the culture bottles were stored in a laboratory setting. Flies obtained from the culture bottles were used in this experiment.

Effect of Creatine Monohydrate Supplement on Longevity in *Drosophila Melanogaster*: Five-day-old virgin females and unmated males from control and creatine monohydrate supplement treated medium were isolated within three hours of their emergence and kept in the lab under the aforementioned conditions. One male and one female of these flies were introduced individually into the mating chamber. If mating does not take place, the pair was discarded. Mated pairs were then moved separately to new vials containing the respective media once every seven days. This practice was carried out till the death of each fly. Twenty (20) replicates per treatment were maintained. The period from eclosion to death was regarded as longevity.

Results

Effect of CrM on Longevity in Female *D. Melanogaster*

In the present study, it was observed the longevity of female flies was significantly different between treatments (control, 2.5%, 5% and 10%) with females maintained on control media having the longest survival time compared to females fed creatine monohydrate supplemented media. The longevity of female *D. melanogaster* decreased with increased CrM supplementation hence the flies fed 10% CrM supplemented media had the shortest survival time. Mean and median values of control and creatine monohydrate treated female flies on longevity in *D. melanogaster* is shown in table 1 while Figure 1 displays the effect of CrM on survivability in female *D. melanogaster*. Table 2 shows the Chi squares values of control and CrM treated female flies on longevity in *D. melanogaster*.

Table 1: Mean and median values for survival time of control and creatine monohydrate treated female flies on longevity in *D. melanogaster*.

Treatment	Mean[a]				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
10%	22.600	.998	20.643	24.557	22.000	1.112	19.820	24.180
2.5%	28.500	.766	26.999	30.001	28.000	1.118	25.809	30.191
5%	24.200	1.204	21.839	26.561	23.000	.742	21.546	24.454
Control	31.850	.990	29.909	33.791	32.000	.894	30.247	33.753
Overall	26.788	.639	25.535	28.040	27.000	.837	25.359	28.641
Estimation is limited to the largest survival time if it is censored.								

Table 2: Chi squares values of control and creatine monohydrate treated female flies on longevity in *D. melanogaster.*

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	34.775	3	.000
Breslow (Generalized Wilcoxon)	35.790	3	.000
Tarone-Ware	36.871	3	.000
Test of equality of survival distributions for the different levels of Treatment.			

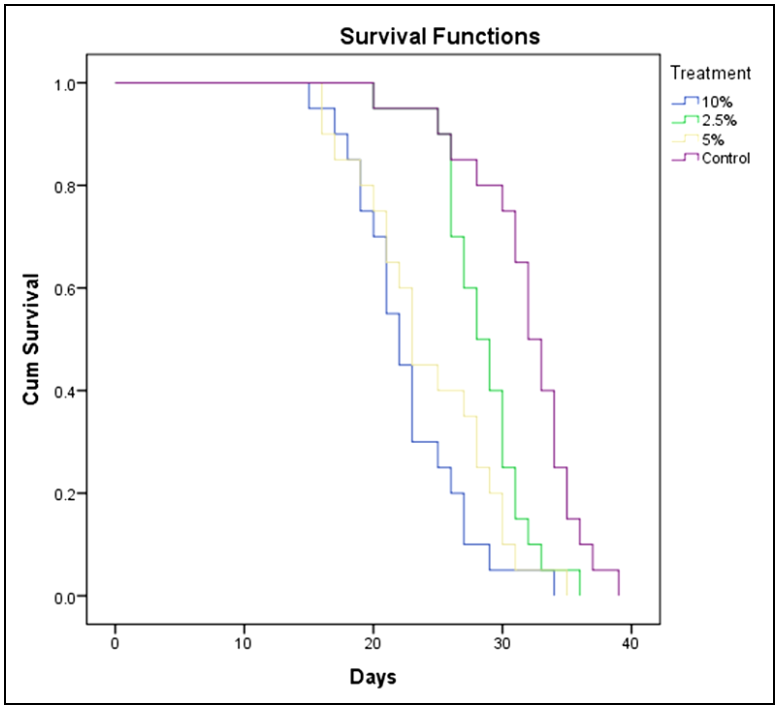

Fig 1: Effect of creatine monohydrate supplement on the survivability of as determined by the Kaplan-Meier survival analysis of female *D. melanogaster*. The graphs represent the survivorship of female flies kept separately in vials (n=80; 20 vials per group). The log-rank test revealed a statistically significant in females (Chi square value = 34.775; df =3; P<0.0001)

The survival function S (t) is defined as the probability of dying at time (t) having survived until that time. The graph of S (t) against t is called the survival curve. The Kaplan-Meir method was used to estimate this curve from observed survival times. Survival curves were compared using a statistical hypothesis test called the log-rank test. i.e., significant variation in longevity was noticed between female flies fed control and creatine monohydrate supplemented media.

Effect of CrM on longevity in male *D. melanogaster*: Male flies maintained on control media had the longest survival period compared to males' maintained media supplemented with creatine monohydrate, it was shown in the current study. Male flies maintained on treatments 2.5%, 5%, and 10% had considerably shorter lifespans than those maintained on control media. The flies fed medium enriched with 10% CrM had the shortest survival times because the longevity of male *D. melanogaster* reduced when CrM supplementation was increased. Table 3 lists the mean and median values for control and CrM-treated male flies on lifespan in *D. melanogaster* while the Chi squared values of the control and CrM-treated male flies on *D. melanogaster* longevity are displayed in Table 4. Figure 2 depicts the impact of CrM on the survivorship of male *D. melanogaster*.

Table 3. Mean and median values of control and creatine monohydrate treated male flies on longevity in *D. melanogaster*.

Treatment	Mean[a]				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
10%	19.850	1.168	17.560	22.140	18.000	1.118	15.809	20.191
2.5%	24.300	1.052	22.239	26.361	25.000	1.112	22.820	27.180
5%	21.900	1.051	19.840	23.960	21.000	2.236	16.617	25.383
Control	28.150	.874	26.436	29.864	29.000	.730	27.569	30.431
Overall	23.550	.618	22.339	24.761	24.000	.994	22.052	25.948
Estimation is limited to the largest survival time if it is censored.								

Table 4: Chi squares values of control and creatine monohydrate treated male flies on longevity in *D. melanogaster.*

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	23.830	3	.000
Breslow (Generalized Wilcoxon)	26.312	3	.000
Tarone-Ware	26.117	3	.000
Test of equality of survival distributions for the different levels of Treatment.			

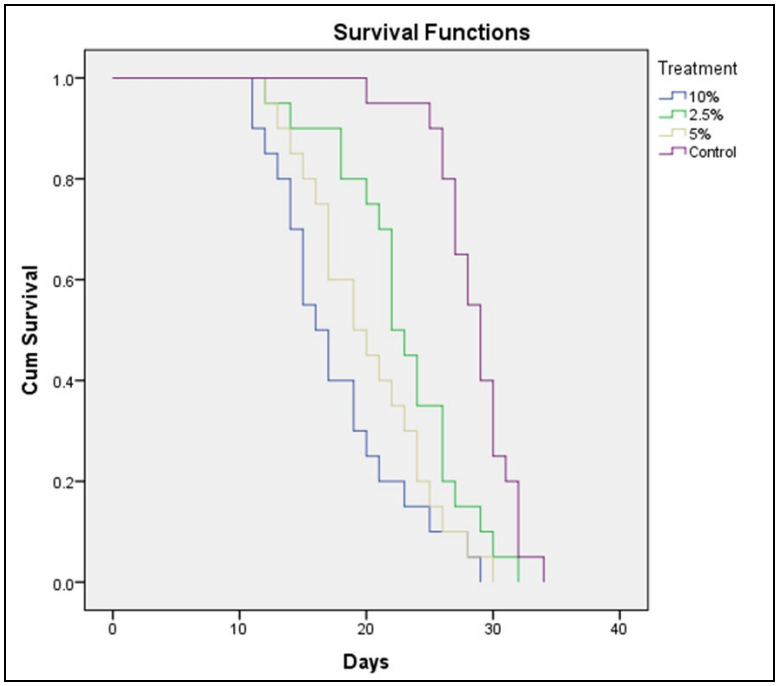

Fig 2: Effect of creatine monohydrate supplement on the survivability of as determined by the Kaplan-Meier survival analysis of male *D. melanogaster*. The graphs represent the survivorship of male flies kept separately in vials (n=80; 20 vials per group). The log-rank test revealed a statistically significant in males (Chi square value = 23.830; df = 3; P>0.0001)

The survival function S (t) is defined as the probability of dying at time (t) having survived until that time. The graph of S (t) against t is called the survival curve. The Kaplan-Meir method was used to estimate this curve from observed survival times. Survival curves were compared using a statistical hypothesis test called the log-rank test. i.e., significant variation in longevity was noticed between control and creatine monohydrate supplement male flies.

Discussion

Differences in longevity are a crucial life history feature because they show how long *Drosophila* live and how much nutrition an organism consumes (Wafa and Krishna, 2015; Alwyn and Krishna, 2015) [1]. In the present study, it was discovered that there was sex differences in longevity of *D. melanogaster* whereby the females had higher longevity than males. This confirms results by earlier studies that in many species, there are disparities between the average lifespan of the sexes, and females often outlive males (Lints *et al*. 1983; Austad and Fischer, 2016) [3]. The reported variations in lifespan between the sexes are frequently inferred from wild populations and attributed to risk-taking behavior, feeding habits, and sexual competition, which may have little to do with sex differences in intrinsic aging rates (Austad and Fischer, 2016) [3]. The sexual selection theory (Trivers 1972) [21] contends that lifespan investment and investment in reproduction are trade-offs that result in sex differences in ROS generation and antioxidant defenses.

The study revealed that flies fed on creatine monohydrate supplemented media had short life span compared to flies fed control media (Figure 1 and 2). This suggests that creatine monohydrate had a significant influence on longevity in *D. melanogaster*. This can be attributed to the nutritional differences in the diet since creatine monohydrate is a protein supplement. Growing evidence from recent studies suggests that the dietary balance of specific nutrients (such as protein or amino acids, carbohydrate) is an important factor in life span and ageing in *D. melanogaster* and other insects (Mair *et al*. 2005; Min & Tatar 2006; Lee *et al*. 2008; Maklakov *et al*. 2008; Fanson *et al*. 2009; Grandison *et al*. 2009a; Dussutour & Simpson, 2012). The study has shown that creatine monohydrate reduce longevity in female and male *Drosophila melanogaster*. This result approved that high-protein diets shorten lifespan in many organisms as stated by Arganda *et al*., (2017) [2].

Conclusion

The study has revealed that creatine monohydrate supplementation reduces its longevity and female *D. melanogaster* have longer life span compared to males.

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