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Sexual Dimorphism in the Effect of a Taurine Supplemented Diet on Life Span in Adult *Drosophila melanogaster*

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ABSTRACT

As the amino acid taurine is being used more frequently in human and animal diets, the exact physiological role and benefit have not been fully elucidated. To determine if taurine can impact long term physiology, we investigated the effects of a chronic taurine supplemented diet using the model organism, *Drosophila melanogaster*. We specifically studied how life span and development are affected. A pairwise comparison of survival curves found a significant difference between male and female fruit flies supplemented with a taurine diet. Another pairwise comparison found a significant difference between taurine and normal fed fruit flies with respect to gender. Fruit fly eggs are sensitive to exogenous taurine concentrations reducing the number of hatched larvae. Present studies indicate chronic taurine provided throughout the life time may be beneficial to life span extension in adulthood.

Key words: *Drosophila*, taurine, aging, life span, development

INTRODUCTION

Taurine (2-Aminoethanesulfonic acid) is a β-amino acid, synthesized *de novo* from the amino acid cysteine by way of cysteine dioxygenase (Stipanuk, 1986). There is increasing evidence regarding taurine as a key amino acid in both vertebrates and invertebrates, responsible for the proper function of a wide range of physiological systems (Oja and Saransaari, 2007). Taurine has been shown to be a vital nutrient in the diet of felines, which are unable to synthesize taurine, for proper development of the retina (Schmidt *et al*., 1977; Knopf *et al*., 1978). Studies have also been conducted in dogs and some species of fish which suggest that taurine supplementation may be necessary in commercial dog and fish food for proper nutrition (Backus *et al*., 2006; Lunger *et al*., 2007). The physiological roles of taurine include absorption of fat, maintenance of proper osmolarity, anti-oxidant capabilities and as a neuromodulator (Huxtable, 1992; Oja and Saransaari, 1996). In captive animals, such as lions and polar bears, taurine is an important part of the diet needed to prevent rickets by aiding in the absorption of fat soluble vitamins (Chesney and Hedberg, 2009; Chesney *et al*., 2009). Taurine acts as an osmotic regulator in several fish and bivalve species, allowing the marine animals to react to changes in salinity (Avella *et al*., 2009; Inoue *et al*., 2008; Hosoi *et al*., 2007).

Research has shown levels of taurine differ in *Drosophila* tissues and in hemolymph (Massie *et al*., 1989; Piyankarage *et al*., 2008). Identification of a taurine transporter, dEAAT2, in

Drosophila has implicated taurine to act as a neuromodulator (Besson *et al.*, 2005). In humans, taurine is the second most abundant amino acid within brain tissue. In the nervous system, taurine can inhibit the firing of neurons by increasing chloride conductance through activation of GABAa and glycine receptors within the nervous system (Albrecht and Schousboe, 2005). Additionally, taurine has been shown to act as a neuromodulator and neuroprotective agent by reducing CaMKII activity, providing protection from epilepsy-inducing agents and preventing excitotoxic damage by activating $GABA_A$ receptors (Junyent *et al.*, 2009, 2010; Louzada *et al.*, 2004). Studies on neural tissue and insulin sensitivity in rats have shown that taurine acts as an anti-oxidant, protecting neural tissues from arsenic-induced oxidation and improving insulin sensitivity in hyperglycemic subjects (Das *et al.*, 2009; Haber *et al.*, 2003). Taurine has been shown to reduce the oxidative stress of the heavy metal contaminant, cadmium, in fish and mice (Kumar *et al.*, 2009; Sinha *et al.*, 2008). The multitude of contributions that taurine makes to physiological functions have established taurine as an important component of life and suggest that taurine supplementation can confer protection from various forms of degeneration.

The processes that ultimately contribute to cessation of life and factors that alleviate degeneration are not extensively understood. While many organisms do not experience senescence as a result of aging, most organisms including *Drosophila*, experience some form of functional decline (Helfand and Rogina, 2003). *Drosophila melanogaster* has been the model system of choice for many life span studies that examine the effects of environmental influences, genetic adaptation and behavioral changes on life span. Life span studies allow a cohort of flies to be studied in order to identify physiological problems that can become evident in a reduced life span or identify any benefit to the organism if the life span is extended. Factors such as energy consumption, energy expenditure and oxidative stress, among many others, can affect the initiation and/or rate of mortality in *Drosophila* (Helfand and Rogina, 2003). The goal of the present study is to examine the effect of taurine, a known anti-oxidant, anti-inflammatory agent, metabolic enhancer, osmoregulator and neuromodulator on *Drosophila* life span and development (Oja and Saransaari, 2007; Zamboni *et al.*, 1993; Haber *et al.*, 2003; Junyent *et al.*, 2009; Murakami *et al.*, 2002; Tsuboyama-Kasaoka *et al.*, 2006).

MATERIALS AND METHODS

Fly stocks and conditions: The wild type strain, Canton S (Bloomington stock number 1), was reared at 23°C on cornmeal-molasses food as normal food or supplemented with the addition of specific concentrations of taurine (Acros Organics). Virgin flies were selected within 8 h of clearing the vials for all studies. Flies were passed and counted twice a week for life span studies unless otherwise specified.

Developmental dose curve: Cages of approximately 200 flies were acclimated to food plates with either normal or taurine supplemented food for at least 3 days. Grape juice plates with yeast paste were used to collect eggs to be transferred to food vials with the appropriate food. Eggs were collected for no more than 2 h each day until 100 eggs were collected for each concentration of taurine. For 18 days, the progress of the eggs was monitored daily to indicate the number of eggs hatched, the number of pupae produced and the number of adults that emerged.

Life span studies: Ten virgin Canton S flies, either randomly or equal numbers of males and females, were placed in cornmeal-molasses food vials. For the taurine supplemented vials, appropriate concentrations of taurine were added to the food mixture upon cooling right before

filling the vials. Twice a week, the flies were counted and transferred to fresh vials throughout the life spans. Median age of flies was calculated by subtracting the number of flies on the current date from the previous date, then multiplying the result by the current number of days. These calculations from throughout the life span were added together and then divided by the total number of flies in the experiment (Dr. Blanka Rogina, personal communication).

Statistical analysis: When comparing multiple treatments, such as in the progeny development study, analysis of variance (ANOVA) was used. Multiple pairwise comparisons were conducted using the Bonferroni correction method for the significance level. This technique adjusts the significance level needed to reject the hypothesis of equality of two treatments by taking the original significance level and dividing by the number of comparisons. This technique preserves the overall error rate at the original significance level (Neter *et al.*, 1996).

For all life span studies, survival analysis was used. Survival analysis was appropriate to study the time to death of the flies with a given treatment. Survival modeling allows the data to be censored. In fact, the data collected was actually interval censored because the event of death was not exactly observed but did occur in some interval of time between the days the vials were examined.

Three different parametric distributions for creation of the survival curves (Weibull, lognormal and logistic) were evaluated for fit to the data. The best fitting distribution was the Weibull distribution. The Weibull distribution is identified by its shape (α) and scale (β) parameters (Cassell and Berger, 2002; Allison, 1995). Once a survival curve was created for each treatment, the shape and scale parameters were statistically tested between treatment groups to determine whether or not there was a significant difference between the treatment group survivorship curves. ANOVA was used to determine if there was a statistical significance between at least two of the treatment survival curves. If the ANOVA was rejected, multiple pairwise comparisons were conducted using the Bonferroni correction to test the difference between the shape and scale parameters of the survival curves. The Bonferroni correction takes the original significance level and divides by the number of comparisons to maintain the experimental error rate. If either the shape or scale was significantly different, this would mean the survival curves were statistically significant. Using ANOVA over the traditional Kolmogorov-Smirnov test was beneficial due to the discrete nature of the data with interval censoring (Cesani *et al.*, 2006; Mentre and Escolano, 2006). The Kolmogorov-Smirnov test should only be used when the data is continuous (NIST/SEMATECH, 2003). Also, the Kolmogorov-Smirnov test does not easily generalize to allow testing of multiple curves (Dasari *et al.*, 2007).

Finally, a two sample t-test was used to determine the significance of the median and maximum ages between normally fed and taurine fed flies.

All analysis were conducted using the statistical software R (R Development Core Team, 2009). The survreg function in R was used to create the survival models. The ANOVA function was used in R to conduct ANOVA.

RESULTS

Effects of taurine concentration on progeny: Earlier study on the effects of taurine demonstrated adults fed 200 mM taurine is lethal to the progeny; however, the exact stage and concentration has not been identified (Massie *et al.*, 1989). We established cages of 200 wild type Canton S mating flies and acclimated the adults to normal food and various concentrations of

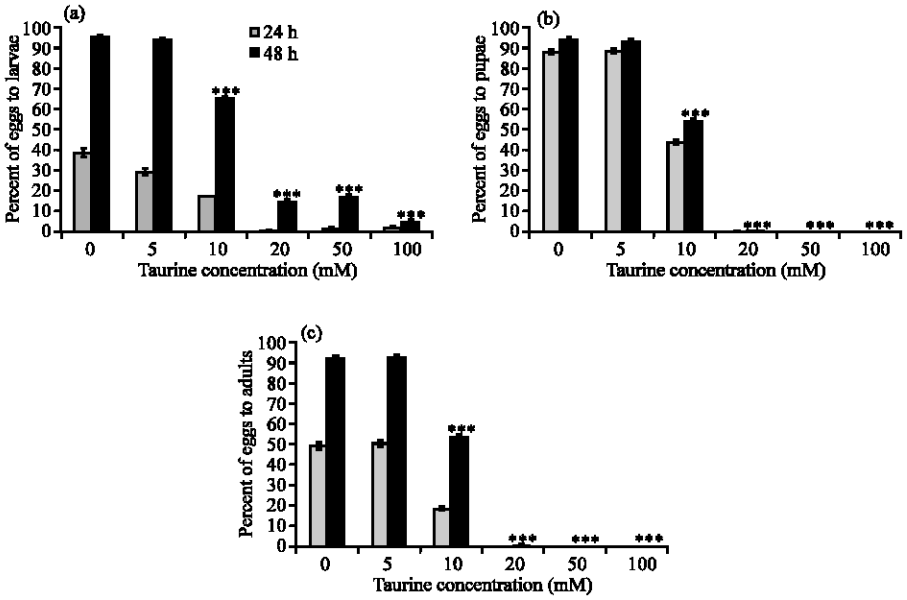

Fig. 1: Taurine dose response of eggs developing into adults. Eggs laid on grape juice plates were transferred to vials of food with 0, 5, 10, 20, 50, or 100 mM taurine and allowed to develop for 18 days. Taurine at concentrations of 10 mM and higher affect the hatching of eggs to (a) larva. Further development of the larvae to (b) pupae and to (c) adults is only slightly affected by the presence of taurine in the food. Two time points are shown to demonstrate early and late time points for each stage. ANOVA and multiple pairwise comparisons used to determine the significance of the late time points, ***$p<0.0001$

taurine for 4 days. After 30 min, twenty eggs were transferred from the grape juice plates to vials containing the appropriate food and checked daily for developmental progress. As shown in Fig. 1a, there is a dose response in the number of eggs that are hatched. Two time points are shown in the figures: an early time point in which a number of larvae (24 h), pupae (8 days) and adults (12 days) were observed in the control treatment and corresponds to normal development of the fly and a later time point in which all of the developmental stages should have been complete (48 h, 11 and 15 days). The early time points show that taurine delays the development of all stages, particularly at 10 mM taurine and higher. As the taurine concentration increased, egg hatching at the later time point was reduced from 97% to less than 20% at 20 mM taurine (Fig. 1a). The number of eggs that developed into pupae at the early and late time points for each taurine concentration shows that 10 mM and greater taurine affects the development of pupae (Fig. 1b). From egg to adults, the lowest concentration of taurine, 5 mM, does not affect the development of adult flies (Fig. 1c). The decrease in the number of eggs hatched, eggs to pupae and eggs to adults was statistically significant for taurine concentrations of 10 mM and higher for the later time points (pairwise t-test, $p<0.0001$). The percentage of eggs developing into pupae and adults is approximately the same as the number of eggs that hatch for 10 mM taurine, suggesting that development beyond the first instar larvae at 10 mM taurine is not affected. Thus, exogenous taurine is most detrimental at the early stage of egg to first instar larva. These results confirm the data reported by Massie *et al.* (1989). We also repeated this experiment providing only normal food to the mating flies in the cages followed by the transfer of eggs to the various concentrations of

taurine. We obtained similar results to our initial experiment, which provided each taurine concentration to the mating adults and the developing eggs, suggesting that the effects of taurine are not due to mating problems (inability to fertilize the eggs) or due to maternal contribution (data not shown).

Life span on taurine-supplemented food: Wild type flies (Canton S) selected within eight hours after enclosion from pupae were collected and culled 10 per vial (total of 10 vials per treatment per trial). A random mixture of males and females were placed in the vials. The flies were fed a cornmeal-molasses food alone or supplemented with 100 mM taurine. Concentrations for taurine were based on the data from the experiments performed by Massie *et al.* (1989) in which it was shown that 50, 100 and 200 mM taurine supplemented in Formula 4-24 Instant *Drosophila* medium did not affect the life span of the wild type strain Oregon R. Using the wild type strain, Canton S, we confirmed that no progeny were produced by adults fed 200 mM taurine and higher; however, the life spans of Canton S flies at 200 mM taurine and higher resulted in premature death after 2 weeks (data not shown). Thus, we continued life span studies using 100 mM taurine. Flies were transferred and counted twice a week over the life span. Red dye was added to the food to allow visual inspection of the fly abdomens for indication that flies were consuming the food. We observed both normal and taurine fed flies consumed similar amounts of the food. Three separate life spans of control and taurine fed flies (n = 3) were independently completed. The median life span for flies fed normal food was 33.68±0.41 (SE) days while supplementing the food with 100 mM taurine the median age was 38.57±0.95 (SE) days. The median age of taurine fed flies is significantly larger than normal fed flies (t-test, p = 0.0111). The extension of life span could especially be seen in the maximum life span (median age of the last 10% of flies) where taurine fed flies lived maximally to 99.98±8.73 (SE) days while normal fed flies lived for 72.56±2.56 (SE) days. The maximum age of taurine fed flies again is significantly larger than normal fed flies (t-test, p = 0.0390).

In order to compare the trend over the entire life spans of normal and taurine fed flies, survival analysis using the Weibull distribution was used. As stated in the Materials and Methods section, the Weibull, lognormal and logistic distributions were investigated for best fit. An example of the fit can be seen in Fig. 2. The Weibull does the best overall fit compared to the other curves because of its performance at the beginning and ending of the life span. It is also a decent fit for the middle section. The logistic curve does an insufficient job at the beginning time points by underestimating the percent alive. The lognormal underestimates events in the middle of the life span. All the life span experiments followed similarly in the fit of the curves to data. Therefore, the Weibull distribution was used throughout the paper to fit survival curves.

Flies fed 100 mM taurine-supplemented food over the course of the life span followed a similar decrease in survivorship as flies fed normal food over the first 30 days (Fig. 3). After 30 days, the decline in the percent of flies alive is steeper in the normal fed flies than taurine resulting in the extension of the life span. ANOVA was conducted and found that at least two of the survival curves were statistically significant in either the shape, scale, or both parameters. Therefore, pairwise comparisons were done using a Bonferroni correction for the significance level of $\alpha = 0.05/$ (30 comparisons) =0.0017. First, it can be seen in Fig. 3, Table 1 and 2 that the three trials of normal (all p>0.02) are statistically the same as are all three trials of taurine (all p>0.0999) meaning that shape and scale parameters are not different within each treatment.

From Table 1 and 2 there is no statistically significant difference between normal and taurine fed flies within each trial (all p>0.0041). However, the small p-value for normal versus taurine

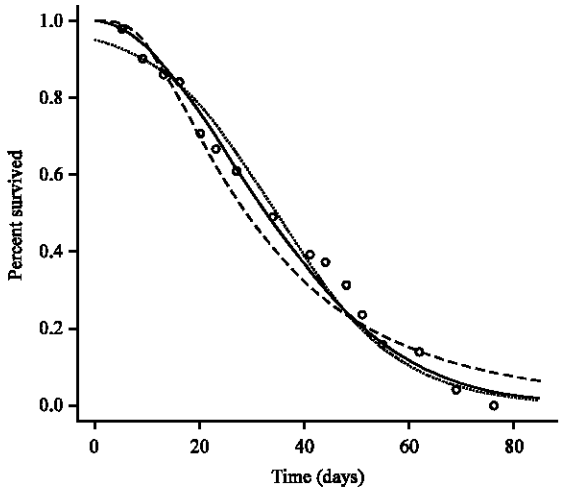

Fig. 2: Graph used to determine goodness of fit for the three distributions, Weibull (thick line), lognormal (solid, thin line) and logistic (dashed line) for the first trial of taurine supplemented flies in the normal versus taurine mixed experiment. The Weibull does the best at both the beginning and the end of the time period. The logistic curve underestimates at the early stages and the lognormal underestimates the distribution in the middle where most of the events occur and it overestimates at the end stages. Therefore, the Weibull distribution was used for all analysis

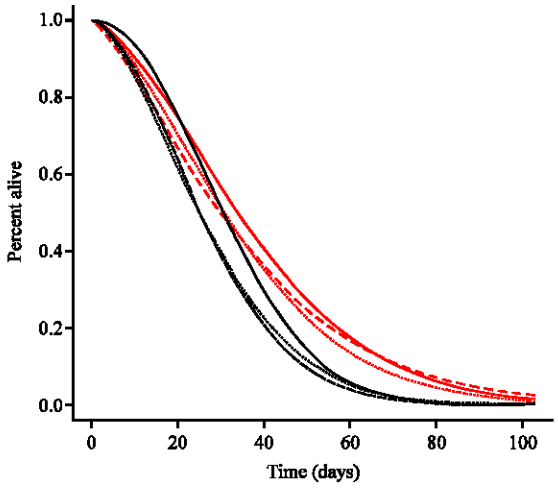

Fig. 3: Fitted Weibull survivorship curves of random mixture of male and female fruit flies fed normal cornmeal-molasses food and flies fed food supplemented with 100 mM taurine. Solid lines represent trial 1, dashed lines represent trial 2 and the dotted lines correspond to trial 3. Normal fed trials are on the left (black lines) of the corresponding taurine fed trials (red lines). There were three independent trials for a total of approximately 300 flies per treatment. Initially, both taurine and normal fed flies followed the usual decline in the number of flies over time until about day 30. After day 30, the average number of taurine-fed flies is more likely to live as compared to the control as shown by the steeper slope in the normal curve

Table 1: The p-values of each pairwise comparison between the means of the scale parameter β for the random mixture of flies fed normal and taurine food

	Normal 1	Taurine 1	Normal 2	Taurine 2	Normal 3	Taurine 3
Normal 1		0.0703	0.0200	0.4016	0.1105	0.5251
Taurine 1			0.0002*	0.3518	0.0035	0.4012
Normal 2				0.0048	0.9169	0.0291
Taurine 2					0.0323	0.9666
Normal 3						0.0742
Taurine 3						

*p-value of 0.0017 or less is viewed as significant after the Bonferroni correction

Table 2: The p-values of each pairwise comparison between the means of the shape parameter α for the random mixture of flies fed normal and taurine food

	Normal 1	Taurine 1	Normal 2	Taurine 2	Normal 3	Taurine 3
Normal 1		0.0248	0.1240	0.0001*	0.0481	0.0267
Taurine 1			0.3664	0.0999	0.9498	0.8441
Normal 2				0.0041	0.4673	0.3183
Taurine 2					0.1402	0.2375
Normal 3						0.8135
Taurine 3						

*p-value of 0.0017 or less is viewed as significant after the Bonferroni correction

trial 2 (scale p = 0.0048, shape p = 0.0041) suggests there is some evidence supporting a difference between taurine and normal fed flies. If not for the Bonferroni correction, normal and taurine trial 1 would also be significant (scale p = 0.0703, shape p = 0.0248). Given the borderline insignificance of the survival curves and the significance of the average median and maximum ages, we believe that taurine may be extending life span, but some confounding factors, such as the concentration, critical period for supplementation and gender, may be masking the effects of taurine.

Life span with a lower concentration of taurine-supplemented food: As noted in our initial experiments recapitulating the work from the Massie lab., with 100 and 250 mM taurine-supplemented food, flies were not surviving long in the 250 mM taurine-supplemented food and were significantly reducing the survival of the progeny. If female flies reduce fecundity due to poor conditions (i.e., toxins in the environment), this can also increase life span. Thus, we wanted to use a lower concentration of taurine that did not dramatically affect fecundity to determine whether it was effective in extending the median and maximum life spans of flies as compared to normal. Following the same feeding regimen as the higher taurine concentration (100 mM) previously used, we repeated the life span experiment using 10 mM taurine (5 vials, 10 flies per vial). At the lower taurine concentration, the median age and maximum age also increased over the normal treatment. The median age for 100 mM taurine fed flies was 35.82 days, 10 mM taurine fed flies is 35.10 days and normal fed flies is 28.80 days. The maximum age for 100 mM taurine fed flies is 81.86 days, 10 mM taurine is 96.50 days and normal is 63.50 days. Both taurine treatments increased both median and maximum age compared to normal. Survival analysis showed no significant difference among all three treatments (ANOVA, p = 0.0970). Again this p-value is borderline for the typical significance level of $\alpha = 0.05$. Due to the borderline p-value, pairwise comparisons were still conducted (Table 3). It appears there is slight evidence that normal fed flies and 10 mM taurine fed flies have different scale and shape parameters (scale p = 0.0290, shape

Table 3: The p-values of each pairwise comparison between the means of the scale parameter β and shape parameter α for the normal, 10 and 100 mM taurine experiment

	Scale component β			Shape component α		
	Normal	Low	High	Normal	Low	High
Normal		0.0290	0.0739		0.0419	0.8386
Low			0.9455			0.0279
High						

*p-value of 0.0083 or less is viewed as significant after the Bonferroni correction

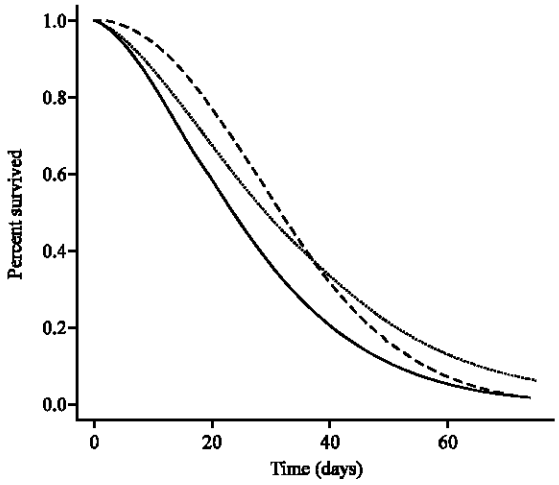

Fig. 4: Fitted Weibull distributions for each treatment group (normal, 10 and 100 mM taurine concentrations). From left to right, the survival curves are normal food (solid line), 100 mM taurine (dotted line) and 10 mM taurine (dashed line). Flies fed normal food follow the typical Weibull curve and show a faster decline in the number of living flies. The two concentrations of taurine shift the curve to the right increasing the survivorship of flies throughout the life span

p = 0.0419). This suggests there is a possible difference in the survival curves. A Bonferroni correction of $\alpha = 0.05/$ (6 comparisons) =0.0083 was used to determine significance. Figure 4 shows the Weibull fitted survival curves of the three treatments, normal, 10 mM taurine and 100 mM taurine. Normal and 100 mM taurine curves look similar in shape, but 100 mM taurine fed flies are shifted to the right, increasing the number of flies surviving throughout the life span. The 10 mM taurine curve takes on a different shape compared to the normal curve. Throughout most of the life span (until about day 60), 10 mM taurine appears to be beneficial to survival, keeping a larger number of flies alive.

Critical period for taurine supplementation: After 30 days, flies fed taurine-supplemented food appeared to have an advantage that allowed the extension of the maximum life span (Fig. 3). Based on this result, we investigated whether a chronic diet of taurine-supplemented food was necessary for the extension of maximum life span or if there is a critical time period (within or after the first four weeks of adulthood) that taurine must be supplemented in the diet to confer the life span extension. As shown in Fig. 3 and 4, chronic taurine-supplemented food (both high and low

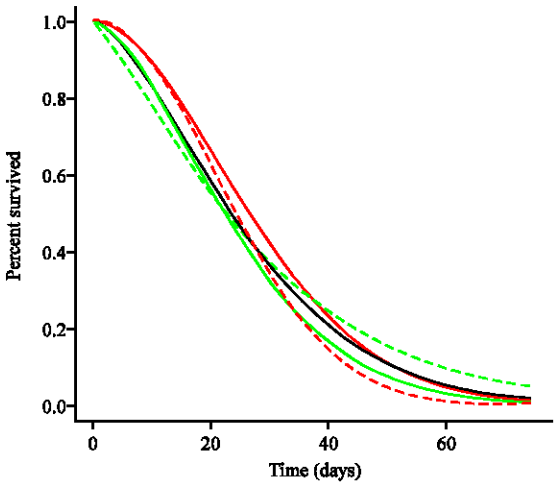

Fig. 5: Fitted Weibull distributions to each treatment group that switches food after 4 weeks (normal-10 mM taurine, 10 mM taurine-normal, normal-100 mM taurine, 100 mM taurine-normal and normal-normal). Solid lines represent normal to high concentration, normal to normal and normal to low concentration (respectively), dashed lines represent low concentration to normal treatment and high concentration to normal food (respectively)

concentrations) compared to normal food extends the maximum life span of the flies. In these experiments, flies were given normal food for the first 4 weeks followed by either 10 mM taurine or 100 mM taurine for the remainder of the life span. The reciprocal experiments beginning with either 10 mM taurine or 100 mM taurine followed by normal food for the remainder of the life span were also conducted.

An overall comparison of normal, normal to 10 mM taurine, normal to 100 mM taurine, 10 mM taurine to normal and 100 mM taurine to normal found no statistical significance among the groups (ANOVA, $p = 0.5200$). A graphical representation of the Weibull survival curves are shown in Fig. 5. All curves look very similar to normal in both scale and shape.

Life spans of mated male versus female on taurine: We also performed life span studies in which the sex of mated flies were taken into account. Vials of 10 flies consisting of 5 females and 5 males were fed either normal cornmeal-molasses food or food supplemented with 100 mM taurine throughout the life span (total of 100 flies per sex). When the flies were passed and counted, the gender of the dead flies were identified and recorded. The median age of normal males was 32.64 days, taurine males was 55.62 days, normal females was 36.34 days and taurine females was 25.18 days. The maximum age for normal males was 64.67 days, taurine males was 119.00 days, normal females was 74.57 days and taurine females was 63.40 days. Both normal males and females have similar median and maximum ages. The most interesting component is that 100 mM taurine dramatically decreases both median and maximum ages of females while the males benefit from exogenous taurine. Taurine fed females also have a median and maximum age less than that of normal females.

Survival analysis of the life spans of normal and 100 mM taurine fed sexes show that taurine fed sexes are affected differently. Testing the equality of all the survival curves was rejected (ANOVA, $p<0.0001$). The survival curves of the life spans show that normal male and female curves

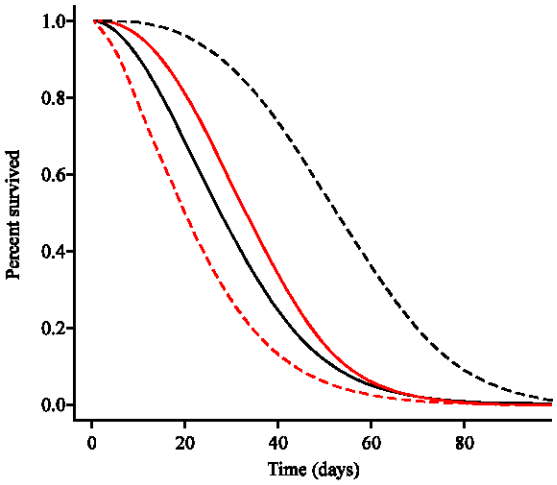

Fig. 6: Fitted Weibull distributions to each treatment group (male-normal, male-taurine, female-normal, female-taurine). Solid lines represent normal food. The bottom dashed line is the female-taurine group and the top dashed line is the male-taurine group. Throughout the entire life span, all treatment groups respond similarly to the control group

Table 4: P-values of each pairwise comparison between the means of the scale parameter β and shape parameter α for each sex on normal and 100 mM taurine food

	Scale component β				Shape component α			
	Normal male	Taurine male	Normal female	Taurine female	Normal male	Taurine male	Normal female	Taurine female
Normal male		<0.0001*	0.1576	0.0230		0.0091	0.1585	0.2132
Taurine male			<0.0001*	<0.0001*			0.1788	0.0003*
Normal female				0.0001*				0.0098
Taurine male								

*p-value of 0.0042 or less is viewed as significant after the Bonferroni correction

are similar (scale p = 0.1576, shape p = 0.1581) while the curve for taurine fed males is shifted far to the right compared to normal males (scale p<0.0001, shape p = 0.0091) increasing the likelihood of survival throughout the life span (Fig. 6). The p-values were compared to a Bonferroni correction of $\alpha = 0.05/$ (12 comparisons) = 0.0042. Taurine decreases the survival of females (scale p = 0.0001, shape p = 0.0098) demonstrated by the shift to the left in the curve compared to normal females (Table 4).

DISCUSSION

As taurine is increasingly being used in the energy drink and sports supplement industry and animal diets, it is imperative to study the long term effects of its usage. To this end, we have utilized the model system, *Drosophila melanogaster*, to investigate whether taurine affects life span and development of progeny. At high concentrations (greater than 200 mM) of taurine, adult fruit flies consuming the supplemented food prematurely died. Upon inspection of red dye added to the food to determine if the flies were consuming the food, control and taurine fed flies had comparable amounts and percentages of flies with positive indicators. Based on our findings, high levels of taurine cannot be tolerated by adult fruit flies.

Observations by our lab and previously reported by Massie *et al.* (1989) have shown that taurine is lethal to developing embryos. We counted the number of larvae, pupae and adults that emerged from embryos placed on food with varying concentrations of taurine. Massie *et al.* (1989) reports that the normal number of adults emerged when exposed to 20 mM taurine or less; however, our data show greater than 5 mM taurine begins to significantly affect the development of the embryos to adults (Fig. 1). Fruit flies are most affected early in development before hatching from the egg cases. We were able to exclude both mating problems and maternal contribution as factors involved in the effect taurine exhibits on egg hatching. Exactly how taurine can pass from the environment through the egg case is not fully understood and requires further study.

Our initial experiments on life spans neglected the balance of genders in each vial using a mixed population. From a series of three independent experiments, the median and maximum life spans of flies fed 100 mM taurine consistently were extended as compared to flies fed normal cornmeal-molasses food (Fig. 3 and Table 1 and 2). We also demonstrated the lower concentration of taurine (10 mM) conferred a similar effect (Fig. 4, Table 3). These findings are in contrast with the work by the Massie lab., which found no effect of taurine on life span in fruit flies (Massie *et al.*, 1989). However, we believe that one reason that the previous lab was unable to detect life span extension by taurine is the difference in wild type strains used. It has been reported that the genetic background difference between the two wild type strains, Canton S and Oregon R, may be responsible for the varying degree of life span extension in dietary restriction experiments (Bhandari *et al.*, 2007). Oregon R flies increased the life span 40% while in the same experiment, the increase for Canton S was 80-90% for life span extension under caloric restriction (Bhandari *et al.*, 2007). Further studies using other standard controls, such as *wg* (wingless), used in aging experiments should be investigated to verify our findings.

The survival analysis curves of the chronically fed taurine flies (Fig. 3) indicated a possible transition phase in which taurine was most noticeably effective during the latter half of the life span. We investigated whether there was a critical period in which taurine supplementation could become ineffective in extending the life spans. Providing either 10 mM or 100 mM taurine before or after 4 weeks did not extend median or maximum ages (Fig. 5). All survival analysis curves for switching from normal food to taurine supplemented food or vice versa are similar to the curve for normal food throughout the life span. The 4 week switch in treatment was chosen based on the initial survival curve that indicated that there was a transition at about day 30. However, it is possible that week 4 is too long to make the switch as it is very close to the median age of flies. Future experiments will investigate earlier week transitions from the treatments.

A possible mechanism of life span extension is dietary restriction in which the amount of calories from protein (yeast for fruit flies) is reduced resulting in life span extension. The results shown in Fig. 5 provide support that dietary restriction is not the mechanism that taurine is working through because the effects of dietary restriction are reversible. When flies are switched from control to calorically restricted food or vice versa, the flies will exhibit a mortality rate associated with the food source within the last 48 h (Mair *et al.*, 2005). If taurine is acting as a deterrent of food consumption (i.e., calorically restricting), in our experiments we would expect that flies fed taurine after the 4 week transition (last portion of life span) would revert to a pattern of life span extension similar to chronically fed taurine. In our experiments, flies fed normal food for 4 weeks then transferred to taurine, did not extend the median or maximum life spans as seen in the chronically fed taurine groups. This provides some evidence, in addition to the similar amounts of red dye

consumed, that taurine is not reducing caloric intake and extending life span through dietary restriction. To confirm that caloric restriction is not how taurine is affecting life span, using mutants that affect metabolism and caloric intake, such as *chico* or *Indy*, in similar studies with taurine supplemented food are necessary.

In the experiments in which gender was taken into account for life spans, both median and maximum ages of females fed taurine were statistically different than the males fed taurine. Females died early in the life span resulting in decreased median and maximum life spans compared with normal fed females and were dramatically different than the median and maximum life spans in taurine fed males. Often the explanation for a discrepancy between genders in aging studies is that females are more attune to possible toxins in the food source and may restrict their diets and the cost of reproduction (Jafari *et al*., 2006; Fowler and Partridge, 1989). The basic theory is that there is prioritizing between reproduction and growth/maintenance depending on the availability of nutrients (Piper *et al*., 2005). The inability to reverse the effects of potential calorically restricted food as shown in Fig. 5 provides some evidence that this is not the case; however, our experiment did not distinguish gender to verify the effects on each sex. Yet, the dramatic decrease in life span seen in the taurine fed females may still be due to passing the threshold of the beneficial calorically restricted diet. There is a discrete amount of caloric restriction that provides an increase in life spans, but reducing too many calories can starve flies (Mair *et al*., 2005). Given the complicated nature of aging in mated females, using virgin females, which already have longer life spans than mated females, may provide better insight into whether taurine can affect longevity.

Present results demonstrate that exogenous taurine may provide age related benefits when taken throughout adulthood. We are currently investigating the possible mechanism of how taurine is extending life spans, most dramatically on maximum life spans. As shown by our results, taurine is most effective provided throughout adulthood (Fig. 3, 4 and 6). This seems to suggest that taurine is working as an anti-oxidant that removes the accumulation of free radicals over time. Intervention throughout the life span keeps the build up of free radicals to a minimum while intervention later in life or for the first 4 weeks of adulthood cannot reverse the damage. There are a few possible ways in which taurine may be exerting an anti-oxidant effect in flies: taurine itself is scavenging free radicals, taurine is activating one or more genes associated with an anti-oxidant pathway such as GSH (glutathione) or taurine may be providing nutritional feedback. As noted from *in vivo* studies, taurine is not effective in removing peroxides or superoxide anions suggesting that it is not likely a free radical scavenger (Aruoma *et al*., 1988). A recent study has linked a decrease in the amino acid, methionine, to life span extension (Grandison *et al*., 2009). Since taurine is indirectly made through a methionine pathway, exogenous taurine may provide a feedback loop to reduce the level of methionine. An imbalance of amino acids in the fruit fly diet, specifically too much methionine, may lead to free radical damage. When methionine is removed from the diet, the accumulation of free radicals may be slowed leading to the life span extension. A possible pathway taurine may be acting on is dTOR (target of rapamycin) which provides a nutritional signal to control growth response through the IR/PI3K (Insulin receptor/ phosphoinositide 3-kinase) pathway (Oldham *et al*., 2000). Another likely mechanism is that taurine activates pathways that can remove free radicals, specifically, through initiation of GSH and Sir2 (sirtuin). Cysteine is a pre-cursor of both taurine and GSH. Additional taurine in the diet may allow more cysteine to be utilized as GSH, which can inhibit the accumulation of peroxides and extend life spans as seen in mosquitoes (Richie *et al*., 1987). Life span extension by activation of

Sir2 using taurine may affect genes associated with metabolism, stress resistance and apoptosis (Frankel and Rogina, 2006). We are currently investigating these possible mechanisms to determine how taurine exerts a beneficial effect in male fruit flies.

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