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Article

The Effect of Different Fructose Diets on the Lifespan of *C. elegans*

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SUMMARY

Sugar, such as fructose, is widely known to be a dietary cause for many health complications including diabetes, heart disease, and even cancer, and is an enticing yet harmful substance if consumed in large quantities. Fructose, typically in the form of high-fructose corn syrup, is the sugar most commonly found in fast foods. Thus, we chose to study the health effects of a high fructose diet on humans through experimentation on *Caenorhabditis elegans*, which are effective model organisms due to their rapid reproduction rate and stability despite variations in environmental conditions. We hypothesized that increasing sugar intake in *C. elegans* will reduce *C. elegans* survival, though moderate amounts of sugar may increase survival. The results show that the concentration of fructose had a significant influence on the survival rate of *C. elegans*. The *C. elegans* receiving 0% and 5% fructose concentration treatments had much higher survival rates than the 15% plates, which had zero surviving *C. elegans* after six days. After statistical analysis, the 5% and 15% plates were determined to yield significantly different survival rates. Thus, there is sufficient data to conclude that diets containing high levels of fructose negatively impact *C. elegans* life, suggesting that diets high in sugars such as high fructose corn syrup are harmful to humans. However, it was not possible to discern any significant difference between the 0% and 5% treatments from the data generated. Further experimentation would be needed to investigate the effects of diets containing a moderate amount of sugar.

INTRODUCTION

Overconsumption of sugar is known to reduce human life expectancy by causing a variety of diseases such as type 2 diabetes, heart attacks, and hypertension, posing a major risk to public health, especially in the United States (1, 2). *Caenorhabditis elegans* is a suitable model organism because 40% of human disease genes are homologous to genes in the *C. elegans* genome (3, 4). As a result, *C. elegans* have been well documented under many controlled conditions and are used in various studies to model systems in the human body, including issues regarding sugar-induced toxicity (5, 6) (5, 6). Thus, examining the effects of sugar on *C. elegans* gives insight on how it affects the human body, providing an ethical way to study human diseases. Previous studies found that high glucose diets in concentrations above 2% or fructose diets in concentrations at or above 10% shorten the lifespan of *C. elegans* (7). Zheng *et al.* discovered that while 555 mM (10% w/v) of fructose decreased lifespan, low amounts of fructose at 55 mM (1%) and 111 mM (2%) actually increased lifespan (8). High doses of fructose increase intestinal fat deposition (IFD) which disrupts the balance of hormones in *C. elegans*, resulting in a shorter lifespan and impaired ability to maintain homeostasis (8). Therefore, high fructose diets may also have harmful effects humans, since the human digestive system shares similarities with that of *C. elegans* (9). These include similar processes of lipid metabolism due to homologous genes for fat storage, making *C. elegans* a good model for human energy homeostasis and metabolic pathways (10, 11). Based on the conclusions made by Zheng *et al.*, we hypothesized that with a diet of 5% (278 mM) fructose concentration, the *C. elegans* lifespan increases in comparison to the worms exposed to the 0% fructose concentration, because the amount of sugar in a 5% concentration is not high enough to generate excessive IFD, instead providing extra energy for the worms to grow (8). Furthermore, we hypothesized that fructose concentrations of 10% (555 mM) or above at 15% (833 mM) would cause the lifespan of the *C. elegans* to decrease due to excessive IFD. In our experiment, we found that, in general, increasing concentrations of fructose decreased *C. elegans* rate of survival. Thus, our results indicate that fructose may have similar effects on humans, supporting claims that higher amounts of sugar can be detrimental to bodily health, generating excessive body fat.

RESULTS

In this study, we tested the effects of different concentrations of fructose on the survival rate of *C. elegans*. This was tested by subjecting the *C. elegans* to 0%, 5%, 10%, and 15% fructose concentrations in a total of twenty separate petri dishes, the concentration of fructose being the independent variable. The experimental control was the 0% fructose dishes which acted as a negative control, establishing the baseline of *C. elegans* survival when they are not treated with fructose.

To account for external variables such as temperature and light exposure, all of the dishes were kept together in the same place so as to be exposed to the same environmental conditions and were measured at approximately the same time every day. To ensure controlled group numbers, only five *C. elegans* were put into each petri dish so that the fluctuations in *C. elegans* population could be better observed and compared each day.

When gathering results, each petri dish was observed

Days After Plating	0% fructose	5% fructose	10% fructose	15% fructose
0	5/5	5/5	5/5	5/5
1	7/36	9/20	8/21	9/18
2	10/22	8/11	12/23	2/21
3	19/22	11/14	14/24	0/20
6	139/146	469/502	41/57	0/8

Table 1. Number of *C. elegans* alive over time over the total number of *C. elegans*. The data graphed in **Figure 1**, which shows how many *C. elegans* were observed to be alive each day (0, 1, 2, 3, and 6 days) for each test (0%, 5%, 10%, and 15%). The individual data points were calculated by finding the sum of the number of *C. elegans* alive on a particular plate in the five trials for each fructose concentration.

Days After Plating	0% fructose	5% fructose	10% fructose	15% fructose
0	100	100	100	100
1	19.4	45	38.1	50
2	45.5	72.7	52.2	9.5
3	86.4	78.6	58.3	0
6	94.5	93.4	71.9	0

Table 2. Average percent of *C. elegans* left alive days after start of experiment. The average percentage of C. elegans alive for each fructose group on each day (0, 1, 2, 3, and 6 days), calculated by dividing the number of C. elegans moving by the total number counted in each plate and averaging the resulting values for each test (0%, 5%, 10%, and 15%) (n = 5).

under a microscope every day to count the number of living *C. elegans* and the total number of *C. elegans* found on each plate, dead or alive. To determine which worms were alive we took advantage of the fact that when stimulated by light emitted by the microscope, living *C. elegans* display negative phototaxis movement, while dead *C. elegans* do not move. We counted the total number of live *C. elegans* for each concentration of fructose daily (**Table 1**, **Figure 1**); the total number of *C. elegans* counted each day was also recorded (**Table 1**). Using the ratio of *C. elegans* alive to the total number of *C. elegans* observed on each dish, the percentage of *C. elegans* alive on each dish was calculated. For each concentration, we calculated the average of the percentage of *C. elegans* alive and the standard error of the percentages each day (**Table 2**, **Figure 2**). We display the fluctuations in population growth and survival of *C. elegans* over time for each fructose concentration (**Figure 1**, **Figure 2**).

Further investigating the statistical meaning of our data, we conducted an ANOVA test. The null hypothesis states that there is no statistically significant difference between all of the fructose groups; the alternate hypothesis states that there is a statistically significant difference between the fructose groups. The test yielded an f-statistic value of 4.1643 and a *p*

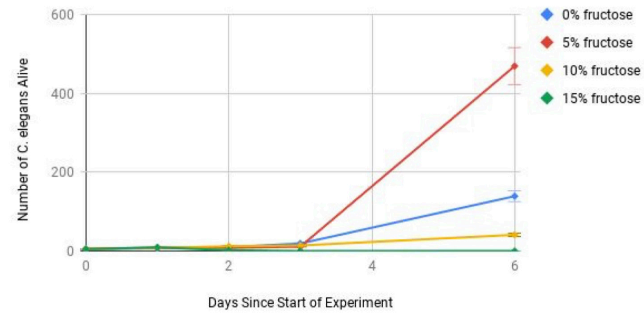

Figure 1. Effect of different fructose concentrations on *C. elegans* population size. The total population of C. elegans for each fructose concentration test (0%, 5%, 10%, and 15%) was observed over time (0, 1, 2, 3, and 6 days).

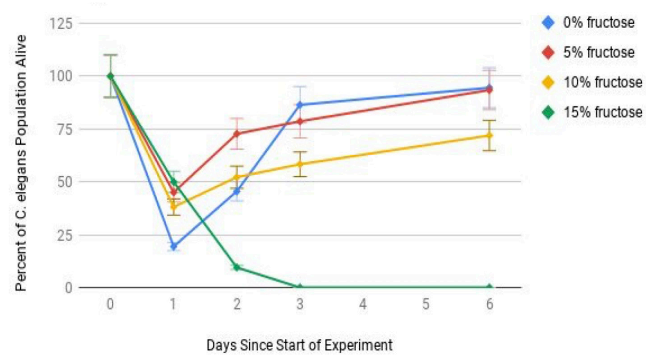

Figure 2. Effect of different fructose concentrations on percent of C. elegans alive. The percent of C. elegans alive in each test over time (0, 1, 2, 3, and 6 days). For each test (0%, 5%, 10%, and 15%), five trials were done and error bars represent standard error.

-value of 0.0309 (**Figure 3**). The calculated *p-value* is less than the critical value of 0.05, rejecting the null hypothesis. Thus, there was a statistically significant difference between the four fructose groups.

To identify which pairs of groups had statistically significant differences, a Tukey honestly significant difference (HSD) test was done (**Table 3**). Pairs 0% and 5%, 0% and 10%, 5% and 10%, and 10% and 15% had *Q-values* 0.8922, 0.5142, 1.4064, and 3.2722 respectively. With values less than the $Q_{critical\ value}$ of 3.46, the Tukey test indicated that there was no significant difference within these pairs. However, the 0% and 15% fructose pair showed a possibility of significant difference with a *Q-value* of 3.7864, and the 5% and 15% fructose pair yielded a significant difference with a *Q-value* of 4.6786.

After the first day, the percentage of *C. elegans* alive in the 5% fructose group was higher (45.0%) than in the 0% group (19.4%) (**Table 2**). However, when the 5% fructose line dipped lower than the 0% line after three days (**Figure 2**), the 0% and 5% fructose tests resulted in survival rates of 94.5% and 93.4% respectively on Day 6, which, according to the Tukey test, are not statistically different. Thus, it appears that adding a moderate amount of fructose to the *C. elegans'* diet may not have a beneficial effect on the survival rate of the *C. elegans*. However, the numbers of living *C. elegans* in the 0% fructose test and 5% fructose test are worth noting and investigating in the future; the 0% fructose test yielded a total of 139 *C.*

ANOVA Summary					
Source	Degrees of Freedom DF	Sum of Squares SS	Mean Square MS	F-Stat	P-Value
Between Groups	3	7561.0419	2520.3473	4.1643	0.0309
Within Groups	12	7262.772	605.231		
Total:	15	14823.8139			

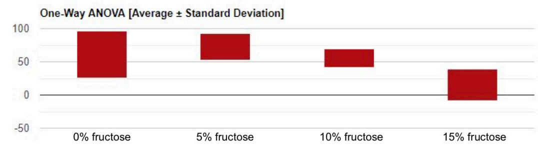

Figure 3. ANOVA test between all groups (0%, 5%, 10%, and 15% Fructose). Displays the ANOVA test done on the data for all of the groups (0%, 5%, 10%, and 15%). The null hypothesis states that there is no difference between the means of the groups. The calculated *p*-value is 0.0309.

Fructose groups	0% & 5%	0% & 10%	0% & 15%	5% & 10%	5% & 15%	10% & 15%
Q-value	0.8922	0.5142	3.7864	1.4064	4.6786	3.2722

Table 3. Tukey HSD Test Between All Groups (0%, 5%, 10%, 15% Fructose). *Q*-values from the Tukey test done on the data for all groups (0%, 5%, 10%, and 15%). The *Q*-critical value is 3.46.

elegans alive, and the 5% fructose test yielded a total of 469 *C. elegans* alive, the 5% fructose group clearly having a much higher resulting population. Looking at the other test groups, the 15% fructose group had zero surviving *C. elegans* by the sixth day, which was quite different from the 71.5% survival rate of the 10% fructose group and was a stark contrast with the near 100% survival rates of the 0% and 5% fructose groups. According to the results of the ANOVA and Tukey tests, there was a significant difference between the 15% fructose diet and both the 0% and 5% fructose diets, supporting that a higher fructose diet leads to lower survival rates. Therefore, the results partly align with our hypothesis; increasing the concentration of fructose in the diet of *C. elegans* decreases survival, though there is insufficient evidence to conclude that moderate amounts of fructose (5%) increase *C. elegan* life.

DISCUSSION

The experiment was designed to test how different concentrations of fructose in the diet of *C. elegans* would affect their survival rate, which was measured by counting the number of worms alive on each plate every day. Prior research indicated that the lifespan of *C. elegans* is correlated with the concentration of fructose in their diet, because the sugar builds intestinal fat in the worms, disrupting homeostasis in their bodies (8). With an impaired ability to maintain homeostasis, the *C. elegans* may be unable to carry out bodily functions effectively, subsequently decreasing their rate of survival. The results of this experiment support our hypothesis that increasing fructose concentrations above 10% lowers survival rate. Low concentrations of fructose were hypothesized to improve survival by providing a low damage energy boost though the results do not support that a 5% fructose diet would necessarily increase survivability.

The ANOVA test confirmed that there was a significant difference in survival rates among the groups. The post-hoc Tukey test indicated that between the groups there was a statistically significant difference in survival rate between the 0% or 5% fructose groups and 15% fructose group, concluding that when fed diets containing over 10% fructose, *C. elegans* survival significantly decreases. On the other hand, there is not enough evidence to indicate that there was a statistically significant difference between the 0% and 5% groups or between the 0% and 10% groups, both with *Q-values* below the $Q_{critical\ value}$. As a result, we cannot conclude that 5% fructose diets increase survival or that 10% fructose diets necessarily result in a significant decrease in survival. These results suggest that high fructose diets, which are lethal to *C. elegans*, may also have detrimental impacts on human health due to the high degree of conserved genes.

However, there are facets of the experimental design that could have caused error. One of the potential sources of error in this experiment was the method of placing *C. elegans* into the petri dishes. By using the inoculating loops, which are too big for accurate worm selection, it is possible that *C. elegans* eggs were carried over into the agar plates, causing unintended variances in the initial number of *C. elegans* in each plate. Furthermore, it was difficult to single out worms of the same size, so worms at different stages of their life cycle were used. However, trouble plating the worms was uniform for all of the experimental groups, so this error does not create bias and does not alter the results of the experiment. This would, however, be a possible explanation for the error bars on the data points that are indicative of variation between the groups. Finally, a factor that could have caused errors in the data is that Days 4 and 5 of data collection were skipped due to our inability to collect data over the weekend, resulting in an incomplete data set.

Another problem arose when analyzing the data regarding the number of *C. elegans* observed at the end of the experiment. The vast difference in the number of *C. elegans* present and the number alive begs the question of how to factor these differences into the statistical analysis. Since there is not one consistent total number of *C. elegans* in each group, the ANOVA and Tukey tests could only be conducted on the percentage of *C. elegans* alive. However, there is quite a large difference between having 57 *C. elegans* left in the 10% group and having 146 left in the 0% group, which is also very small in comparison to the 502 left in the 5% group (**Table 1**). Though both the 0% and 5% groups had near 100% survival rates, the difference between the numbers of living *C. elegans* observed in these two groups leads to uncertainty regarding whether a 5% fructose diet does indeed increase growth rates.

Therefore, future experiments can benefit from ensuring that the stated variables are securely controlled so as to minimize bias in the resulting data. It is worth investigating how to keep the total number of C. elegans constant and how to compare survival rates in a statistical analysis while taking into consideration the number of C. elegans in total at the end of the experiment. Thus, this experiment could be revised to better gauge the effects of different fructose diets on *C. elegans*. Furthermore, the optimal fructose concentration in the *C. elegans* diet for the highest rate of survival could also be tested by using smaller increments of fructose concentration, for example testing one percent instead of five percent, so as to have more precise observations. Due to the homology between *C. elegans* and humans, this information can be used to advance research in the medical field, allowing for a better understanding of what levels of sugar and fructose are beneficial or harmful to humans.

MATERIALS AND METHODS

To set up the experiment, we repeatedly microwaved nematode agar (Fisher Science) for approximately 30 second intervals until a liquid solution was obtained. While the agar was melting, we acquired 20 small petri dishes and split them into 4 groups of 5 dishes, each group labeled with the concentrations being tested: 0%, 5%, 10%, and 15% fructose (Scholar Chemistry). We labeled the five petri dishes in each group individually with the numbers 1 through 5 to keep track of trials. Then we measured 2.5 grams, 5 grams, and 7.5 grams of fructose for the 5%, 10%, and 15% solutions respectively. After measuring out the fructose, we mixed each amount of fructose with 50 mL of water and 1 gram of nematode agar to create four diluted solutions, one solution containing only water and agar for the 0% test group. We then poured these solutions into their respectively labeled plates and left the dishes to cool for one day with the lids on to prevent contamination. We obtained the *C. elegans* through Dr. Gabel at Boston University School of Medicine. After the agar solutions cooled and solidified, we placed five live *C. elegans* onto each plate using inoculating loops under microscopes, and sealed the petri dishes with parafilm to be stored at room temperature on a lab bench. We distinguished live *C. elegans* from dead *C. elegans* by their movement after slightly agitating the plates, movement indicating life. In the following days, we counted and recorded the number of live and dead *C. elegans* on each plate daily. After the experiment, we conducted an ANOVA test and post-hoc Tukey test on the data for statistical analysis.

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