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The Effects of Buckminsterfullerene with Nutritive Oils on Mice

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ABSTRACT: Oxidative damage is one of the recognized contributors to cell and animal aging. In contrast, antioxidants are natural substances that work to prevent or at least delay this type of cell damage, incorporating the production of free radicals and oxidation. Concerning this study, we will explore the antioxidant Buckminsterfullerene (C_{60}) to associate these positive effects with C_{60}. This interdisciplinary analysis aims to integrate the studies of Chemistry and Biology to perform a meticulous experiment on how the efficacy of nutritive oils on cell longevity and regeneration is impacted when mixed with Buckminsterfullerene, precisely on the mice strain C57BL/6J. Combining C_{60} with salmon oil and olive oil creates five separate study groups, including a control group. The main findings were that the Salmon Oil C_{60} group contained the mice with the highest number of months lived on average, with the most significant percentage increase in longevity compared to the control group (Dry Kibble). The antioxidant successfully maintained a healthy mouse for a more extended period, which holds implications for extending the human lifespan, given further research.

KEYWORDS: Biomedical and Health Sciences; Molecular Biology; Biogerontology; Oxidative stress; Buckminsterfullerene.

Introduction

Aging is a process that occurs in all organisms as time goes by. With the advent of anti-aging research and focus, attention has been put on discovering molecules and compounds that can reverse or prevent the aging process.

Organisms comprise billions of cells, each with its own structure and proper function. As they age, these cells (excluding neurons, cardiomyocytes, skeletal muscle cells, and red blood cells) divide into new cells, allowing for a cell cycle to be repeated until programmed cellular death, also known as apoptosis. Cellular death relies heavily on two components: free radicals and reactive oxygen species (ROS). Free radicals and ROS are self-reliant chemical species that contain an unpaired electron in one of their atomic orbitals.[1] They are understood as vastly unstable and reactive molecules with the power of accepting or donating electrons— withholding the performance of a reductant (accepting element) or oxidant (donating element). These potent molecules aim to attack lipids, nucleic acids, and proteins found in cells— causing the cell to undergo homeostatic disruption, leading to a faster path toward cellular death.[1] As the rate of cellular death increases, cellular regeneration decreases. These species are formed as by-products of metabolic processes in cells.[2] An example of a metabolic process that produces ROS and free radicals as a by-product is cellular respiration, which occurs in the mitochondria and demonstrates how these species can be internally generated.[3] When they "attack" macromolecules found in cells, ROS undergoes the process of oxidation, resulting in oxidative damage. The outcome is an increase in the accumulation of oxidative damage in the aging of cells. Conversely, the degeneration of cells can be slowed using antioxidants, compounds that inhibit oxidation. Antioxidants, also known as cell reinforcements, are natural substances that prevent or at least delay cell damage, including reducing free radicals and oxidation.[4] They are found in various foods, such as vegetables, fruits, and dietary supplements. The research being conducted revolves around the antioxidant C_{60} known as Buckminsterfullerene. Our study will establish conclusions about the effects of nutritive oils with and without added C_{60}.

The average mouse's lifespan is 18-24 months, roughly two years, based on mice in laboratory settings.[5] As longevity is presently being researched with mice, there have been studies in which mice have doubled their lifespan when given the C_{60} molecule.[6] For instance, eight Russian scientists contributed to a four-week experiment where a dose of one to ten mg/kg body weight of C_{60} was orally administered to mice, resulting in changes in the parameters of the mice.[6] These changes included a decrease in liver weight and an increase in glutathione reductase activity. Glutathione reductase is an enzyme encoded in our genes that protects from free radicals and hydroperoxides.[7] However, the difference between our research and the previously published work of scientists in the field is that we will be testing the impact of the C_{60} molecule implemented in a mouse's meal plan and nutritive oils on mice. The oils that will be used are Salmon Oil and Olive Oil. Although there has been research on C_{60}'s and Olive Oil's effect on longevity, we repeat the experiment to confirm the results. In a study by eight scientists at the University of Paris, rats were orally administered C_{60} dissolved in Olive Oil, specifically 0.8 milligrams per milliliter (mg/ml) at repeated doses.[8] They concluded that not only does the C_{60} expand lifespan, but Olive Oil does not cause chronic toxicity.[8] As for Salmon Oil, there has not been research on the combination of Salmon Oil with C_{60} on longevity. We chose to use Salmon Oil specifically as opposed to

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other supplements because this specific fish oil is "loaded with omega-3 fatty acids," which play an influential role in easing inflammation, protecting the sustainability of the heart, and improving brain function.[9]

Methods

This research takes place in a high-school setting, with help from our in-house college faculty, using research conducted from 2018 to 2021. Mice (Figure 1) are ordered from The Jackson Laboratory through JAX Mice, specifically, mice from the mice strain C57BL/6J. This mice strain refers to the genetic combination of the mice; C57BL/6J are genetically modified to be less susceptible to audiogenic seizures and have a low bone density.[5] Additionally, this specific inbred strain is widely used in biological experiments. Upon arrival, the mice are separated into five groups according to the food administered: Dry Kibble, Salmon Oil C_{60}, Salmon Oil, Olive Oil C_{60}, and Olive Oil. Dry Kibble represents the control group. They are housed in tanks filled with aspen (Figure 2). Mice are fed 15% of their body weight, except when severely overweight or underweight; therefore, food percentage is altered. The healthy weight of a male mouse of this strain is between 25 and 35 grams, while female mice range between 20 to 30 grams.

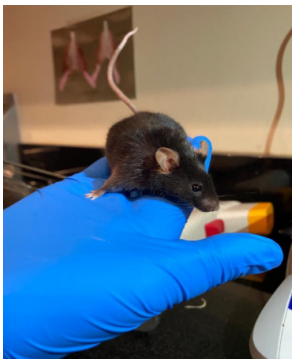

Figure 1: Healthy adult mouse (C57BL/6J) appearance.

Figure 2: Habitat set-up.

Buckminsterfullerene was incorporated into the food through a process of precise measuring: 9375 grams of Dry Kibble was mixed with 0.25 grams of C_{60} and 312.5 grams of the oil, either Salmon Oil or Olive Oil; such a small amount of C_{60} was used because previous studies have shown that low concentrations are more effective for the extension of life.[8] For the groups without the C_{60}, only the Dry Kibble is mixed with the measured oil. First, C_{60} is solubilized in oil and put in a spray bottle. The Dry Kibble is divided into eight trays. Then, one person sprays the mixed oil four times on their tray and passes down the bottle; while the others are spraying, that person is mixing with their gloved hands. To ensure that each piece is getting the same amount of oil and C_{60} after one round of spraying is completed, each person repeatedly passes down a handful of their food to mix with the others; this allows for an equal distribution of oil, accounting for any differences in spraying between researchers. The different treatment groups are stored in labeled, air-tight containers, as shown in Figure 3.

Figure 3: Demonstration of food supply

Although wild mice are nocturnal creatures, the laboratory mice are kept in controlled-light settings from 6 a.m. to 6 p.m., which permits behavioral observation. The categories recorded are their weight in grams (g), their temperature in Celsius (°C), their food intake, as well as their food left over from the previous day, and their water intake in milliliters (mL). In addition, a notation is taken according to the observation of any abnormalities seen on the mouse if, for instance, there is balding anywhere on the body or reddening of appendages, nose, toes, etc. Their activity level is measured in terms of low, medium, or high and written in the notebook. The normal activity level for both female and male mice is generally medium, where they are seen climbing onto their cages, running around, or just walking constantly. A low activity indicates that the mouse is frail: not moving, mostly sleeping during the day, and only getting up to either drink water or eat their supplemented food. Lastly, high activity indicates they are highly active: running around the cage quickly and losing weight due to constant exercise. This information, both quantitative and qualitative, allows the causal-comparative research method to be implemented, therefore permitting a comparison between age and appearance to be made.

With proximity to death, some signs determine whether a mouse is dying, starting with hair loss. Then, low activity, low body temperature, and a decrease in weight can also be noted. When any of these are observed, the mouse is put under a heat lamp with their food placed inside to facilitate their eating ability. However, because the study aims to test C_{60}'s ability to prolong the lifespan, the researchers take minimal meaningful measures to prevent natural death.

Filtering

To effectively conclude results from our pool of mice on natural mouse longevity, we filtered our existing sample pool for unnatural and natural deaths. First, we focused on mice that had died from 2018 to 2021 to conclude from the most relevant results and the current mouse strain. Next, our process included filtering out mice determined to have died from unnatural causes that would skew longevity data. Without this, the predictive model would instead pull from unnaturally low lifespans that were not caused by the dependent variables. For our research, an unnatural death was defined as the death of an adolescent mouse, any mouse under six months of age, due to extenuating circumstances. For example, these include mental factors, such as depression due to the separation of mice from their mother, or physical pre-existing conditions, such as sei-

zures, hemorrhoids, and self-harm. In addition, we filtered out mice with low activity, an indicator of bad health, which would be a confounding variable in determining the effectiveness of C_{60} on longevity.

Limitations

The data reported herein should be interpreted in the context of some limitations. The uneven sample size of mice per food group could have affected the study findings. A larger sample of mice would provide a more thorough distribution of data points, making the data a more accurate representation of life span. This inherent uneven distribution directly affected our findings by necessitating filtering and a stratified random sample to portray our data accurately. Given the researchers are in high school, we acknowledge the transition between researchers, as one graduates and another enters, may affect the mental health of mice. An unfamiliar smell or handling method may present an inherent instability that holds possible implications on quality of life, directly impacting longevity. The high school age of researchers also limited the availability of scientific journals that this study could be compared to and based upon, as many relevant research papers are behind paywalls or inaccessible due to restricted institutional access.

Results and Discussion

In total, there were 68 mice in our population to choose from. After conducting the stratified random sample, data from 55 mice were inputted into our predictive model, and results were concluded from this sample pool. These mice are part of a more extensive study conducted at our high school; however, the mice chosen for our study are randomly selected to ensure that the whole population is represented without bias. All the mice in our data died between the years 2018 to 2021. Therefore, their data was collected from when they turned four weeks old until the day of their death.

Based on the data collected, there is a clear relationship between the type of food given to mice and the number of days they lived (Figure 5). Even though the mouse with the most days lived was under the Olive Oil C_{60} treatment (Drake), the Salmon Oil C_{60} group contained the mice with the highest number of months lived on average, approximately 32 months. The Salmon Oil and Olive Oil groups followed in effectiveness, with an average lifespan of 30.6 and 30.5 months, respectively. Although the nutritive oils alone had a significant impact on the average lifespan of the mice, it is evident that Salmon Oil combined with C_{60} had the most significant percentage increase in longevity when compared to the control group; the mice lived a whole month longer under C_{60} treatment than the nutritive oils alone on average. This can be attributed to the previously mentioned fact that Salmon Oil is loaded with omega-3 fatty acids, whose properties aid in several factors contributing to the mouse's longevity and C_{60}'s antioxidant benefits. First, however, a stratified random sample was conducted on our entire mouse pool of 68 mice to eliminate selection bias and effectively view the effects of nutritive oils. The strata were the specific experimental groups, such as Olive Oil, Salmon Oil, Olive Oil C_{60}, Salmon Oil C_{60}, and our control, Dry Kibble. After determining our strata, we assigned a number to each mouse and utilized a random number generator to select which mice were inputted into our models randomly. The predictive model, based on linear regression, was then pulled from these randomized strata to accurately define the effects of these groups on mouse longevity. Throughout our years of research, we have encountered many different types of mice with natural and unnatural diseases/disabilities, such as proneness to seizures, tumors, and macular degeneration. Macular degeneration is a genetic condition that results in a degenerated macula; in other words - blindness. One mouse under the C_{60} treatment, Rata, was born with macular degeneration in one eye. She was put on the C_{60} treatment approximately six weeks after birth. It showed promising results in Rata's behavior, as she was very active and maintained a healthy weight throughout her life. This suggests that the inclusion of C_{60} into her diet might have improved her quality of life.

The control group consists of 18 mice. The average number of months lived by the Dry Kibble group is 28 months, slightly more than two years. These mice had stable, healthy weights, allowing them to live past the one-year mark and reach their second year; this means that if this study were correct, C_{60} mice would have to live past the three-year mark to demonstrate some longevity. This is supported by the Salmon Oil C_{60} data values, which increase a mouse's average lifespan by 59%.

In the Salmon Oil group, the longest living mouse is Caroline, with 1093 days; this is nearly three years— that is about six months more than the average life of a Dry Kibble mouse. The average number of days lived by the Salmon Oil C_{60} mice is 971 days, around two years and eight months, four months longer than the Dry Kibble group—signifying a causal- comparative analysis between the effects of C_{60} and the days lived. All mice in the Salmon Oil C_{60} group endured healthy weights their entire lifetime, indicating that their deaths were only due to the peak of old age. There is only so much oxidative stress that Buckminsterfullerene can eliminate; the fact that it has allowed for the increase of approximately three months in these mice indicates that the amount of oxidative stress in the body has been reduced each year, allowing for a couple more days to be lived every month.

There exists a significant difference in months lived between Salmon Oil/Olive Oil mice (approximately the same impact according to the negligible difference in average increased lifespan) and Dry Kibble mice, but there is a clear difference between Salmon Oil C_{60} and Salmon Oil/Olive Oil; the data reveals C_{60} extended the lifespan of the mice observed. With the conclusions from the Salmon Oil C_{60} mice and the Olive Oil C_{60} mice, a new inference can be made indicating an expansion of longevity using the antioxidant Buckminsterfullerene, supporting our hypothesis. As predicted, the use of Salmon Oil also contributes to the mouse's lifespan and increases it, which is also supported by the data in the graph (Figure 4). Ultimately, results show that Salmon Oil C_{60} had the most positive effect on lifespan. Based on the combination of these results, one can infer that the lengthening effect observed in Olive Oil C_{60} mice is attributed to the C_{60} treatment instead

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of the oil treatment. On the other hand, Salmon Oil C_{60} mice seemed to outperform this group and the others because both the oils showed an increase in days lived, and when combined with C_{60}, maximum results were achieved.

Implications on Humans

Products are experimented on mice before humans, not only because of the ethical guidelines involved with drug testing but because of their genetic similarity. Humans, like mice, also undergo oxidative stress and have free radicals roaming in their systems. The antioxidant would serve to help eliminate these toxic molecules, in turn expanding an organism's lifespan. In a world growingly obsessed with youth and health, the medical and pharmaceutical fields are captivated by the search for an answer to the widespread demand for life-prolonging substances; humans are very interested in being able to live longer as mortality is not a concept that is thought of positively. By incorporating C_{60} and Salmon Oil into their diets, humans may be able to live longer. Because the Salmon Oil C_{60} group reached a 12% increase in lifespan in the experiment, if results on humans were to directly correlate with this pattern, their lifespan could potentially surpass the 84-year mark; however, it should be noted that an exact correlation between humans and mice is unlikely. Therefore, the pragmatic implications of these findings are promising if the eventual goal is sheerly increasing life length.

■ Conclusion

BuckminsterFullerene is a spherical 60-carbon molecule that behaves as an antioxidant. Oxidative stress, the process in which cell division causes free radicals to be released within the body, is considered one of the leading causes of aging. Antioxidants like C_{60} can absorb these free radicals from an organism's body, thus slowing down the aging process. To test the capabilities of this molecule along with nutritious oils, oil-diluted C_{60} was introduced to the diet of experimental mice. Through a quasi-experimental approach, 55 mice were divided into five groups varying in food and treatment type: Dry Kibble, Salmon Oil, Salmon Oil C_{60}, Olive Oil, and Olive Oil C_{60}. Due to this molecule's previously mentioned antioxidant properties, we expected the mice receiving C_{60} treatment to have significantly longer lives on average than the other groups; however, this was partially supported by the findings as the data revealed different surprising nuances.

The results of this data were evaluated using the meta-analysis method to consider the length and the quality of their lives under the treatments. This method is demonstrated by analyzing multiple data sets and conclusions across all five mice samples and experimental groups. The data revealed Salmon Oil C_{60} as the most effective supplement in prolonging the mice's lifespans. Additionally, it was shown that there is no significant difference between the average days lived by the Salmon Oil group and the Olive Oil group. Therefore, both can be concluded to be effective in extending the lifespan of a mouse when fed the nutritive oils regularly. Using the predictive linear regression model, it was concluded that there was an approximate average 12% increase in lifespan for the mice treated with Salmon Oil C_{60} compared with the control group. Alternatively, the other groups, Salmon Oil and Olive Oil C_{60} saw an 8.5% and 4% lifespan increase, respectively. This indicates an approximate 4-month increase in lifespan for mice treated with Salmon Oil C_{60}. Salmon Oil was the next leading group, with an approximate 2.5-month increase in lifespan. This is visualized in Figure 4 below.

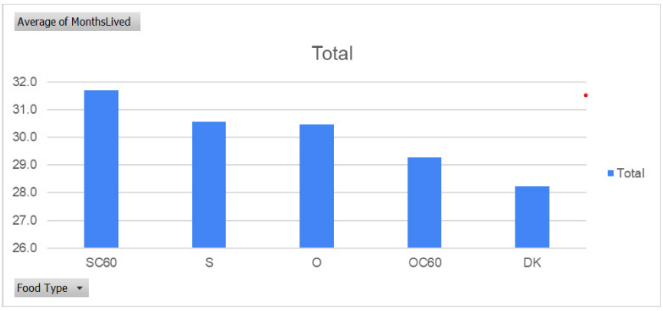

Figure 4: Bar graph of average of months lived per study group. The experimental food groups (Salmon Oil C_{60}, Salmon Oil, Olive Oil C_{60}, Olive Oil, and Dry Kibble) are represented by the x-axis. The average months lived per each group is represented by the y-axis.

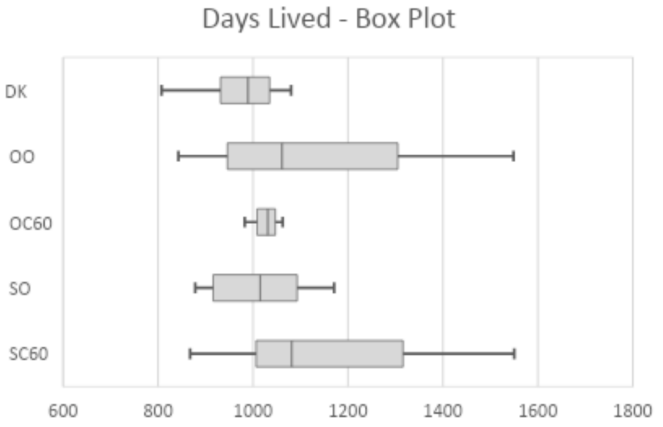

Figure 5: Box plot of average days lived per study group. The standard deviation for days lived in Dry Kibble: 124.7488143, Olive Oil: 197.3034887, Olive Oil C_{60}: 56.73329416, Salmon Oil: 98.67269126, Salmon Oil C_{60}: 248.1741324. The median days lived is Dry Kibble: 931.5, Olive Oil: 946.0, Olive Oil C_{60}: 1008.5, Salmon Oil: 916.0, Salmon Oil C_{60}: 1006.5.

Therefore, we conclude that Salmon Oil C_{60} treatment is the most effective in increasing lifespan and ensuring longevity in mice. Furthermore, when comparing Salmon Oil C_{60} with mice treated with just Salmon Oil, there is a full one-month increase in lifespan. Thus, the antioxidant was more effective in maintaining a healthy mouse longer.

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