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A Comprehensive Review of Dietary and Nutritional-based Therapeutic Approaches for ALS

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Abstract

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that causes patients to progressively lose their motor function. This study reviewed research conducted on transgenic mice that have a human SOD1 transgene with mutations that replicate the physiological symptoms of ALS. One key approach that is used to extend the lifespan of ALS patients lies in nutritional and dietary management approaches, given that ALS patients tend to experience rapid weight loss and metabolic instability as the disease progresses. In this paper, the effects of the ketogenic diet and the Deanna Protocol are analyzed with regards to increases in mice motor performance and longevity. Transgenic mice put on the Deanna Protocol and mice on the ketogenic diet both experienced statistically significant increases in longevity and motor performance as compared to the baseline results of mice on a standard diet. While these results may seem promising, due to the nature of the differences between disease development and progression and the varying effects of the aforementioned diets between mice and humans, further research is still needed to conclude that the same diet-related benefits lie in human ALS patients as well.

Keywords: Neurodegenerative Diseases, Amyotrophic Lateral Sclerosis, Dietary Interventions, Transgenic Mice

1. Introduction

Neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS), have long been a focus of contemporary research. ALS is a neuromuscular disease that mainly affects upper and lower motor neurons responsible for controlling voluntary movements. Belonging to a broader group of motor neuron disorders, ALS is caused by the deterioration and death of motor neurons, which extend from the brain (upper motor neurons) to the spinal cord (lower motor neurons) and to muscles throughout the body. As they deteriorate, neural messaging from the brain to the muscles is severed, resulting in muscle weakening, atrophy, and twitching. Muscle weakness or stiffness are common early symptoms of ALS, and patients gradually lose the ability to speak, eat, move, and even breathe, as all voluntary muscles are affected. Patients subsequently experience muscle atrophy as the disease progresses as well as high metabolic rates, leading to rapid weight loss and eventual malnourishment.

To date, there are only two drugs approved by the

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U.S. Food and Drug Administration (FDA) for the treatment of ALS. The first of these drugs is Riluzole, sold under the brand name Rilutek. Riluzole was approved by the FDA in December 1995 and works to prevent the release of glutamate, which is one of the key causes of ALS when produced in excessive quantities. Based on several clinical studies, riluzole has been shown to decrease mortality rates and increase lifespans in some ALS patients. The second drug approved by the FDA to treat ALS is called edaravone, sold under the brand name Radicava. Edaravone is much more recent than riluzole, as it was approved by the FDA in May 2017, and it works to relieve oxidative stress that may cause motor neuron death.

However, as drug prices have remained high and accessibility to drugs has remained low, there has recently been a flurry of interest related to therapeutic, non-drug treatments for diseases like ALS (Morgan and Kennedy, 2010). About 5,000 people are diagnosed with ALS annually, with as many as 16,000 in the United States living with the disease at any given moment. The mean survival time after being diagnosed is three to five years, and there is currently no cure for the disease (Czaplinski, et al., 2006). Rather than addressing the underlying causes of ALS, current treatments generally serve to alleviate symptoms and increase patient longevity as much as possible with a focus on regulating patients' metabolic activity.

Studies show that the majority of ALS patients undergo metabolic changes, such as hypermetabolism, that lead to shorter survival and a faster functional decline (Steyn, et al., 2018). Hypermetabolism is defined as a significant increase in the body's metabolic activity. Although the origin of this metabolic dysfunction in ALS patients is unknown, research suggests that impaired mitochondrial function, present in motor neurons and muscles of these patients, may lead to the development of this condition (Muyderman and Chen, 2014). Due to the increase in resting energy expenditure caused by the shift in metabolic function, ALS patients have shown a decline in their body mass index (BMI). Not all ALS patients experience hypermetabolism; however, those who do tend to have a lower motor neuron score, indicating motor neuron degeneration, than ALS patients who are normo-metabolic (Ferri and Coccurello, 2017).

BMI is a measure of tissue mass based on height and weight, and individuals are classified as underweight, average weight, overweight, or obese. Studies have shown that individuals with lower BMIs have a higher risk of developing ALS. ALS patients generally have a very low BMI because they lose a significant amount of weight due to muscle atrophy from disuse and because they are generally unable to consume enough calories to maintain their weight. As such, a potential therapy to prolong the lifespan of ALS patients could be based on high-calorie diets and nutritional management in order to promote weight and metabolic stabilization (Dardiotis, et al., 2018).

Currently, there is no cure for ALS, and such nutritional-based therapeutic treatments would likely be used to improve ALS patients' quality of life and extend their lifespans rather than treating the disease itself and reversing its progression. This report will review the effectiveness of two existing nutritional-based approaches to treating ALS: the ketogenic diet and the Deanna Protocol.

The ketogenic diet consists of eating high-fat, low-carb foods, depriving the body of carbohydrates and forcing it to enter a simulated state of starvation in which the body uses fat to produce ketone bodies, which can be used for energy by cells (Swink, et al., 1997). The diet was originally created to treat childhood epilepsy in the early 20th century and its use continued for around two decades before antiepileptic drugs became popular and the use of the ketogenic diet declined (Wheless, 2008). However, in recent years, it has become increasingly popular and is being hailed as a potential treatment for a variety of other neurological conditions and disorders apart from epilepsy, including ALS.

While on the ketogenic diet, patients enter a state of ketogenesis, a biochemical process in which ketone bodies are produced that normally occurs during the night or when dieting/fasting. These ketone bodies are produced from fatty acids and amino acids that are broken down and then converted into acetyl-CoA through beta-oxidation. Ketone bodies are a form of energy used when the body is low on its supply of glucose. Ketones are produced

when the body burns fat for energy because it does not have enough readily available carbohydrates to fuel the body. Ketone bodies are produced primarily in the mitochondria of liver cells and, once produced, they are stored as fatty acids. Some examples of ketone bodies are acetone, acetoacetate, and beta-hydroxybutyrate.

Another dietary-based treatment option for ALS is the Deanna Protocol, a nutritional supplement that targets cell metabolism (ALSUntangled, 2013). Given that ALS is characterized by the death of motor neurons which can be attributed to an excess of glutamate, the Deanna Protocol consists of a multitude of supplements that work against glutamate excitotoxicity. The Deanna Protocol works to deliver AKG (arginine alpha-ketoglutarate) to cells, thus serving as an alternative energy source that the mitochondria can use to keep cells alive (Simplesa, 2018). Similar to the ketogenic diet, the Deanna Protocol does not work to cure ALS; rather, its goal is to improve patients' quality of life and increase their longevity.

This review will examine the effectiveness of the ketogenic diet and Deanna Protocol for ALS treatment, and it is anticipated that these diets will improve the quality of life and slow down motor neuron death of ALS patients, thereby increasing their lifespan.

2. Inclusion and Exclusion Criteria

The clinical studies analyzed in this review were carefully selected to ensure the collection of the most accurate and generalizable results. Generally, any studies that showed bias, did not focus on diet or nutrition, or had any confounding variables such as weight or drug treatments, were not included in this review. More stringent requirements were used for criteria like mouse type, where only studies analyzing two specific transgenic mouse strains (SOD1-G86R/G93A) to minimize inconsistencies caused by different strains of mice responding differently to treatments. Included below is a table detailing the inclusion and exclusion criteria used for this review.

Table 1. Inclusion/Exclusion Criteria

Inclusion	Exclusion
Mice Strain (SOD1-G86R/G93A) SOD1, a gene located on chromosome 21, was first implicated in the development of ALS in 1993. This was the first ALS gene to be identified and the G86R and G93A mutations of the SOD1 gene have been studied in transgenic mouse models (Pansarasa, et al., 2018). The SOD1-G86R and G93A mice are used to study neuromuscular disorders such as ALS because the mutation causes these mice to express neurodegenerative behaviors similar to those experienced by ALS patients (Matsumoto, et al., 2006).	Biases Studies with any form of bias that could have potentially impacted the collected data and results of the experiment(s) conducted were not included.
Mice Gender Male mice are primarily used and preferred over female mice in mice studies as it is a common perception that the hormonal cycle of the females may result in data variability (Smit, 2017). Similar to the menstrual cycle experienced by women, female mice undergo a similar process called the estrous cycle in which mice experience changes in hormonal concentrations. However, this dependence on male models unfairly affects the understanding of disorders and diseases in women. Since ALS affects both men and women equally, the hormonal	No or Minimal Mention of Diet/Nutrition Studies that did not have a focus on analyzing dietary/nutritional-based treatments were not included in this report.

differences in males and females are important to consider when evaluating the efficacy of potential treatments and future research conducted using mice models should not be restricted to male mice alone. (Rozenbaum, 2019).	
Survival Test For quantitative data, only research that kept track of and tested the longevity and mean survival of the mice on the different nutritional and dietary plans was included.	Additional Supplementation/Drugs Studies in which the transgenic mice were tested on any additional supplementation, drug treatment, or physical therapy regimen were not included.
Mice Age Younger mice were favored in the analyzed studies, and all mice were euthanized after the studies were completed.	Overweight/Underweight Mice Studies analyzing overweight and underweight mice were not included in this review to ensure the accuracy of lifespan and metabolism-related measurements taken during the studies. However, such studies should be considered carefully in future reviews, as potential treatments may impact patients of different body types in different ways.
Recency of Studies For the most relevant and up-to-date information and data, only clinical studies conducted after 2003 were included in this report.	

3. Results

In the first study, conducted by researchers at the University of South Florida, 48 male SOD1-G93A mutant transgenic mice were used to test the effects of Deanna Protocol-based and ketogenic diets on disease progression. As stated earlier in the inclusion/exclusion criteria, only male mice were used to minimize data variability due to periodic hormonal concentration shifts in female mice. In this study, four main groups were being analyzed: a control group (mice on the standard diet), mice on the ketogenic diet, mice on Deanna Protocol-based supplements, and mice on the ketogenic diet with Deanna Protocol-based supplements. Motor function was assessed using the tests listed in the table below, and food consumption, weight, and age were all noted throughout the course of the experiment.

In this experiment, survival lengths of all three groups were longer than that of the control group, demonstrating that both the ketogenic diet and the Deanna Protocol have positive effects on lifespan extension. Using the quantitative data included with the study, a relative risk value of 7.03 was calculated between mice on the ketogenic diet with Deanna Protocol-based supplements and mice on the standard diet.

Table 2. Authors and Demographics for Study 1

Authors	Demographics
Ari, et al., 2014	14 B6SJL-Tg (SOD1-G93A) mutant transgenic mice from the Jackson Laboratory. Food consumption and body weight monitored 3 times a week. Animals were received at 9 weeks of age, while treatment and a one-week training period began at 10 weeks of age. Experimental period started at 11 weeks of age from when tests were performed weekly until animals were euthanized. Groups: Standard Diet (SD; Control, n=13); SD+DP (n=12); Ketogenic Diet (KD, n=11); or KD+DP (n=12). Accelerating Rotarod Test, Grip Test, Hanging Wire Test performed once weekly to assess motor function.

This relative risk value represents the ratio of the probability of mice on the Deanna Protocol combined with the ketogenic diet living longer than the baseline age of 125 days versus the probability of mice on the standard diet without any additional treatments living longer than 125 days. Similarly, a relative risk value of 8.07 was calculated between mice on the standard diet with Deanna Protocol-based supplements and mice on the standard diet, suggesting that Deanna Protocol-based supplements may have a more beneficial effect on patients when combined with a standard diet as opposed to the ketogenic diet, which had a calculated relative risk value of 4.00 when compared with the standard diet.

In the second study, conducted at the Université Louis Pasteur, the effects of a high-fat diet (modeled after the ketogenic diet) compared to a standard diet was tested using transgenic male mice with the G86R murine SOD1 mutation (Dupuis, et al., 2004). The high-fat diet consisted of regular chow, which is a high fiber diet with many complex carbohydrates and fats from a variety of vegetables, supplemented with 21% butterfat and 0.15% cholesterol (Warden and Fisler, 2008). Every ten days, body mass was recorded, and every five days, food intake was recorded. Once the mice could no longer roll over after ten seconds of being pushed to their side, they were euthanized and their time of death was recorded. Following euthanasia, the body composition of the mice was measured and analyzed.

The results of this study demonstrated that the high-fat diet was correlated with a 20% extension of the mean survival of the mice. Additionally, mice on the high-fat diet generally exhibited better motor performance than the control mice until their time of death. As predicted, the high-fat-fed G86R mice showed an increase in body mass and the retroperitoneal and epididymal fat pad mass. The study's findings suggest that in addition to improving metabolic status, a high-fat/ketogenic diet may also serve to enhance motor neuron function, allowing the mice to live longer while improving their quality of life.

In the third study, conducted at the Icahn School of Medicine at Mount Sinai, experiments were performed to test the effectiveness of the ketogenic diet as a novel therapeutic treatment for ALS. SOD1-G93A transgenic mice were tested in this study, with one group being fed standard laboratory food while the other was fed food according to the ketogenic diet (KD). This study measured motor performance, survival, and motor neuron count, while also tracking blood ketone levels to verify that the mice on the ketogenic diet had elevated blood ketone levels.

Table 3: Authors and Demographics for Study 2

Authors	Demographics
Dupuis, et al., 2004	Male SOD1-G86R mutant transgenic mice obtained from the animal facility at the Université Louis Pasteur. Male SOD1-G93A mutant transgenic mice obtained from The Jackson Laboratory. Body mass was recorded every 10 days, and food intake was measured every 5 days. Mice were isolated at 4 weeks of age, and the regimen began at 6 weeks of age. Body composition was measured by biochemical analysis of carcasses. Mice were killed when they could no longer roll over within 10 seconds of being pushed on their side, and this time point was used as the time of death.

Table 4: Authors and Demographics for Study 3

Authors	Demographics
Zhao, et al., 2006	SOD1-G93A transgenic mutant mice were used in this study. Experimental mice were fed according to the human ketogenic diet. Blood ketone levels were measured in the KD-fed mice to ensure that the diet was producing the intended effects in the experimental mice. Motor performance in mice was measured using the rotarod test.

In the KD-fed mice, blood ketones were more than 3.5 times higher than the control group, indicating that the diet was working as intended. Additionally, the KD mice lost half of their motor

performance 25 days later than the mice in the control group, suggesting that the ketogenic diet may be able to slow the degradation of motor performance. Throughout the study, weight was measured three times, but there was no significant difference in weight between the two groups of mice: the standard fed mice had a mean weight of 23.5 g compared to 24.6 g in the KD fed mice. At the conclusion of the study, motor neurons were counted in the lumbar spinal cord. The KD-fed mice had considerably more motor neurons in the ventral horn when compared to the control group (9.382 ± 1.125 vs. 6.826 ± 0.607; ($p = 0.03$)), suggesting that the ketogenic diet may have protective effects that serve to slow down motor neuron death. An increase in motor performance was also observed in the experimental mice: the last standard-fed mouse failed the rotarod benchmark cutoff score of 50% 25 days before the last KD-fed mouse failed to achieve the same cutoff score, indicating that increased motor performance may be associated with the ketogenic diet.

Table 5: Authors and Demographics for Study 4

Authors	Demographics
Ari, et al., 2017	30 male B6SJL-Tg (SOD1-G93A) mutant transgenic mice obtained from the Jackson Laboratory. Two experimental groups were used: LOW (88% standard diet, 12% Deanna Protocol supplements) and HIGH (79% standard diet, 21% Deanna Protocol supplements). Body weight of all mice was measured at the beginning of the study and the conclusion of the study. When the mice reached an age of 112 days, the neurological score, a scale of 0-4 measuring the motor ability of the mice's hind legs, of each mouse was calculated and they were subsequently euthanized. Postmortem metabolomic profiling was performed on all mice to determine metabolic differences between control and experimental mice that could be associated with the DP-based intervention.

In the fourth and final study, conducted at the University of South Florida, the effects of the Deanna Protocol on 30 SOD1-G93A transgenic mice were analyzed. The control group was fed standard rodent chow (6.2% fat, 18.6% protein, 75.2% carbohydrate), while the experimental group was divided into two subgroups: LOW and HIGH, corresponding to the size of the dose of Deanna Protocol supplements each group was given. The LOW group was fed the standard diet with 12% consisting of Deanna Protocol supplements by weight, while the HIGH group was fed the standard diet with 21% consisting of Deanna Protocol supplements. At the end of the study, the body weight and neurological score (described in the table below) of all mice was recorded and analyzed.

This study affirmed the results of the other studies and concluded that a Deanna Protocol-based diet is associated with improved mitochondrial energy metabolism and influences the formation of specific metabolites that produce tangible benefits for motor function and performance. Ultimately, given that this study was able to identify the specific biochemicals and metabolites that responded to the Deanna Protocol, these outcomes serve to provide a more comprehensive understanding of potential metabolism-related therapeutic targets for future drug-based approaches for ALS treatment (Ari, et al., 2017).

4. Discussion

In this review, the effects of the ketogenic diet and the Deanna Protocol in the SOD1 mouse model were analyzed. While both seem to be worthwhile for further study, in particular, the effects of the Deanna Protocol are relatively difficult to study because most patients ingest dietary supplements and vitamins that may interfere with the study.

ALS patients frequently experience metabolic disruptions, and as a result, there is a need for treatments that have the ability to stabilize patients' metabolism and weight. Because the ketogenic diet is high in fat and low in carbohydrates, it mimics the effects of fasting and puts the body in a state of ketosis in which the body uses fat instead of carbohydrates for energy. During this process, the body produces ketone bodies from fat for use as an energy source, stabilizing insulin levels and

metabolic rate. The efficacy of several such approaches to ALS treatment is currently being tested.

The Deanna Protocol has a similar outcome as the ketogenic diet for ALS patients; however, the methodology is different. The Protocol consists of taking a cocktail of effective supplements that prevent glutamate excitotoxicity and mitochondrial dysfunction, both part of ALS pathology. The Deanna Protocol is a type of natural metabolic therapy that provides energy to dying nerve cells, thus preventing glutamate release and, therefore, muscular dystrophy. Although the supplementation portion of the Deanna Protocol is the critical component, the complete treatment includes exercise and massages. The Protocol has been studied in conjunction with the ketogenic diet, as examined in Study 1, supporting that the two treatments are complementary. While this review focused on the ketogenic diet and Deanna Protocol, further research should be conducted to determine the effectiveness of other existing therapeutic and nutritional-based treatments for ALS.

It is increasingly important to develop, research, and analyze therapeutic-based approaches to ALS treatment because of the lack of universally effective drug-based treatments, the high cost of purchasing the two available drug-based treatments, and the relatively lengthy process of obtaining FDA approval for new drugs. As mentioned earlier, currently, there are two drugs approved by the U.S. FDA for ALS treatment: namely, Radicava (edaravone) and Rilutek (riluzole). Edaravone produces a yearly cost of approximately $145,000 and corresponding treatment infusion sessions cost approximately $1,000, proving unaffordable for most, even with health insurance. A 1999 study measuring the cost-effectiveness of riluzole concluded that the lifetime survival gain was about 2.3 months per patient, with the incremental cost around $12,000, thus producing an annual cost of $62,609 (DiMasi, 2000). Although the study was performed in 1999, drug prices have risen substantially since then, making for an even more unfavorable cost-effectiveness ratio of riluzole versus care without riluzole. Usage of drugs such as riluzole and edaravone should not be considered the first resort because their effectiveness applies to a very limited subset of people and may result in several serious side effects, including contusions, gait disturbances, headaches, and unusual bleeding. As such, ALS patients often look towards dietary or nutritional-based treatment options to avoid the high risk and cost associated with the approved drugs that prove to be unaffordable for the majority of ALS patients. As new drugs continue to be developed to treat the causes of ALS, the availability of low-cost, easily accessible dietary interventions for the treatment of ALS must expand to help such patients.

5. Conclusion

Across the studies, diets based on both the Deanna Protocol and the ketogenic diet (and high-fat diets in general) were effective in increasing the motor neuron performance and lifespan of SOD1 transgenic mice. Treatments based on these diets could revolutionize the way in which medical professionals approach ALS treatment by allowing patients to decrease their dependence on costly drugs like riluzole and edaravone that are not universally effective in favor of less costly and more effective therapeutic, dietary-based approaches. However, while these results are promising, the studies reviewed focused on analyzing the efficacy of potential dietary therapeutics in transgenic mice, and in order to confirm that the same benefits lie in humans, further studies must be conducted on human patients. Larger-scale clinical trials should also be conducted to minimize potential errors arising from the relatively small sample sizes used in the reviewed studies and determine whether these benefits exist in larger populations as well. If proven to be effective in ALS patients, dietary-based treatments such as those described in this review could also be used to treat other neurological diseases whose mechanisms, development, and progression are similar to that of ALS.

Exercise has also been researched as a potential therapeutic approach to ALS. Studies have shown that mild and moderate endurance exercises have positive effects on ALS patients, while vigorous endurance exercises seem to have detrimental effects. When studied in mice, high-intensity endurance exercise significantly hastened the onset of motor neuron deterioration in male SOD1-G93A mice.

Swimming-based training usually delayed the onset of ALS in these mice, while running-based training in the same study showed no improvement (Tsitkanou et al., 2019). Even if there is evidence that supports the advantageous effects of exercise on ALS patients, it is likely not a sufficient intervention alone to significantly improve patients' quality of life and lengthen their lifespan, as repetitive physical activity can cause muscles to lose strength due to the overworked motor neurons. Often, pharmacological treatments for ALS need to be used alongside exercise. However, as these studies were tested on mice, they are not sufficient for drawing conclusions about the effect of exercise on human ALS patients. As such, further research is needed to conclude whether similar effects of exercise lie in human ALS patients as well.

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