Let the Mice Eat – Understanding Aging Through Dietary Research in Animals

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Introduction and Program Acknowledgement: The Lamming Lab for the Molecular Physiology of Aging is a research team established by Dr. Dudley Lamming in 2014. We investigate the role of diet and metabolism in aging and age-related diseases.

This article is a brief foray into the intersection of nutritional science and aging research. Healthy aging is an urgent research topic in modern medicine. Our overarching goal is to lengthen the period of life when you are physically fit, mentally sharp, and disease-free. A central pillar that governs the aging process is your metabolism, which is an endlessly complex phenomenon. Fortunately, research indicates that one of the strongest contributors to good metabolic health is fully within your control – and that is your dietary habits and your choice of what to eat.

I am delighted to contribute this article to the program "Sharing UW-Madison Postdoctoral Scholarly Research with Non-Science Audiences," sponsored by the Wisconsin Initiative for Science Literacy (WISL). Thanks to the dedication of the WISL staff, I am able to bring my scientific interests and explorations to a wider audience. My passion as a scientist is not just to advance research, but also to share its wondrous discoveries with diverse communities.

If you are like me, chances are high that you were recently taking strolls through the grocery aisles while pondering the everlasting question "*what should I eat*?"

Regardless of your ethnicity, socioeconomic background, or geographical residency, food plays a large role in all of our lives. Dietary habits are an ingrained part of human culture and they frequently dictate the rhythm of what we do, the people we spend our time with, and even how we feel. It should come as no surprise to you that the study of diets, formally called nutritional science, also brings large groups of scientists together. As academics, we similarly obsess over the questions of "what should I eat?", "*how much* should I eat?", and even the provocative "*when* should I eat?" Over the past decades, these topics of investigation have led to several breakthroughs in our understanding of what is considered optimal nutrition. However, there are still a lot of questions to be answered and a long way to go before we can safely apply this knowledge at the population level.

The University of Wisconsin-Madison houses several labs that focus on how the different components of our diets and our eating habits affect our well-being. I am a postdoctoral researcher in the Lamming Lab for the Molecular Physiology of Aging. One of our dedicated areas of focus is to understand how dietary proteins regulate health and longevity. Because metabolism is a whole-body process, we often utilize animal models to investigate the benefits or the detriments of a variety of diets and this article will touch on the many advantages of this approach. We will also discuss some common considerations of performing a well-designed dietary study, how researchers utilize shorter-lived animals to investigate aging, and some findings that have interesting implications on common (mis-)conceptions of our food choices.

The first question you may ask is "why is nutritional research important?" Our body works hard to keep many physiological factors at a balanced level. This includes maintaining blood sugar, fueling the flight and fight response, replenishing strength, and fighting off infections or other abnormal states when necessary. All of this requires energy. Because of this, as you may have guessed, our choices in food are some of the most direct ways that we influence our bodily functions.

This is the fundamental reason why dietary research is important. There are also many specific examples. Here are three you may already be familiar with:

Specialized diets are necessary for those with allergies or rare genetic disorders. Do you know someone who is allergic to peanuts or gluten? Severe food allergies significantly dampen the quality of life and can become life threatening. Even more dangerous are rare genetic disorders that cause metabolic deficiencies in processing certain nutrients; these require specialty diets low in select molecules to avoid a toxic build-up. An example of this is Maple Syrup Urine Disease, which is caused by an inability to metabolize specific proteins. Left untreated, a build-up of unprocessed nutrients can lead to seizure, coma, or death. Fortunately, these severe genetic mutations are routinely screened for and there are promising gene therapies under development to restore these deficits. Dietary research allows us to better understand our bodies and mend diseases when they do occur.

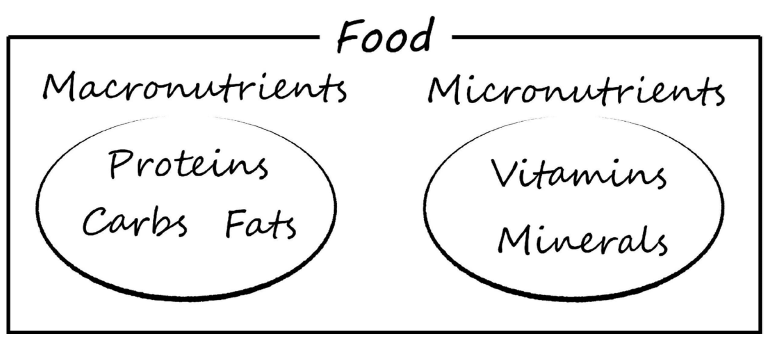
Poor diet is a prominent risk factor for many common illnesses. Heart attack, one of the leading causes of death in the United States, remains inextricably associated with obesity, diabetes, and overconsumption of a Western diet (a stereotypical unhealthy diet excessively high in fat and sugar). These factors can cause poor cardiovascular health, which can then very easily lead to many co-morbidities. An example of this is Alzheimer's disease, which is highly connected with the dysregulation of blood sugar levels and insulin sensitivity. Many refer to this mode of Alzheimer's disease as "type 3 diabetes". By avoiding poor dietary habits, you improve the contents of your circulating blood, leading to better vascular health and reduced risk for chronic health issues. Dietary research, when applied at the population level, allows us to get a bird's-eye view of foods that lead to disease development and the foods that prevent them.

Eat well, feel well. Food is fuel for the body. Every day we face different challenges in life, which equate to different dietary needs. The foods we eat directly contribute to our energy level, hormonal balance, and even mood. By understanding the type of meal that each of us needs, we can use that information to enhance our livelihood. In athletes, optimizing nutrition is a common practice. For example, runners ingest hearty plates of pasta or other starchy meals leading up to a marathon to maximize their energy storage. Similarly, your daily eating habits, including the amount of food you eat and the timing of your meals, can have a great influence on your energy level throughout the day. For example, if you are looking to avoid the urge for an afternoon nap, you may find a lighter lunch to be more suitable. While the ideal meal plan is dependent on the individual, research data can help guide you in the direction that would best suit your lifestyle.

There are endless things to consider in the interactions between our dietary habits and our everyday life. Anyone can benefit from information that helps us better understand how our bodies respond to different types of foods, meal timing, and meal sizes. As a society, dietary recommendations and the labeling of foods as healthy or unhealthy can have a direct impact on the well-being and disease trends of the community. This makes dietary research a high priority for public health officials. Because it is so important to understand the role of diets in our lives, it may interest you to know how scientists carry out dietary research.

Nutritional science – the basics

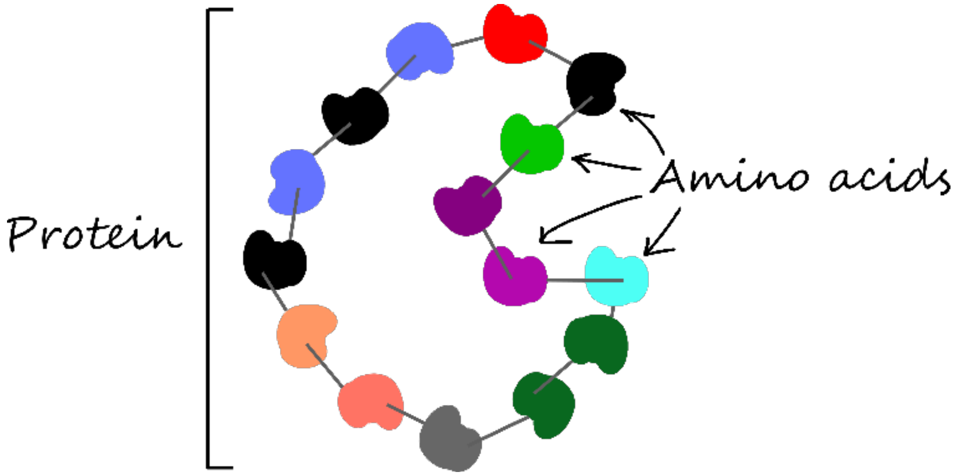
I came to the University of Wisconsin-Madison to study the interaction between aging and dietary proteins. Protein is one of the many complex components present in all foods. I am interested in proteins because our bodies have developed extensive sensory and regulatory mechanisms for some proteins but not for others – but why the favoritism? This screams out to me that something very interesting is happening biologically, and this type of observation serves as the starting point for many of our scientific inquiries. To appreciate this, I would like to first give an overview of the different dietary components. Broadly speaking, there are two main categories of nutrition called macronutrients and micronutrients. Macronutrients include carbohydrates, fats, and proteins. “Macro-“ means big, which means we consume and utilize them in large amounts. On the other hand, “micro-“ means tiny; micronutrients include vitamins and minerals that we eat in fairly trace quantities. In both of these categories, many are considered *essential* nutrients, which is a concise way of saying if you do not eat enough of them, you are going to be in a state of malnutrition. There are also *non-essential* nutrients, which our bodies can create by converting other molecules if there are not enough of them in the diet.



Dietary protein as a class serves several important biological functions and contains many sub-categories within itself. In general, proteins are microscopic workhorses responsible for many vital functions that we commonly associate with being alive. For instance, the protein myofibril forms your muscles, a class of proteins called digestive enzymes break down food, and an unimaginably complex orchestra of different proteins synthesize your consciousness in the form of sensations, perceptions, and memories. In short, you need to ingest a certain amount of total protein in your diet in order to maintain your biological functions, or meet your dietary requirements.

Now this is the most interesting part for me - not all dietary proteins are essential.

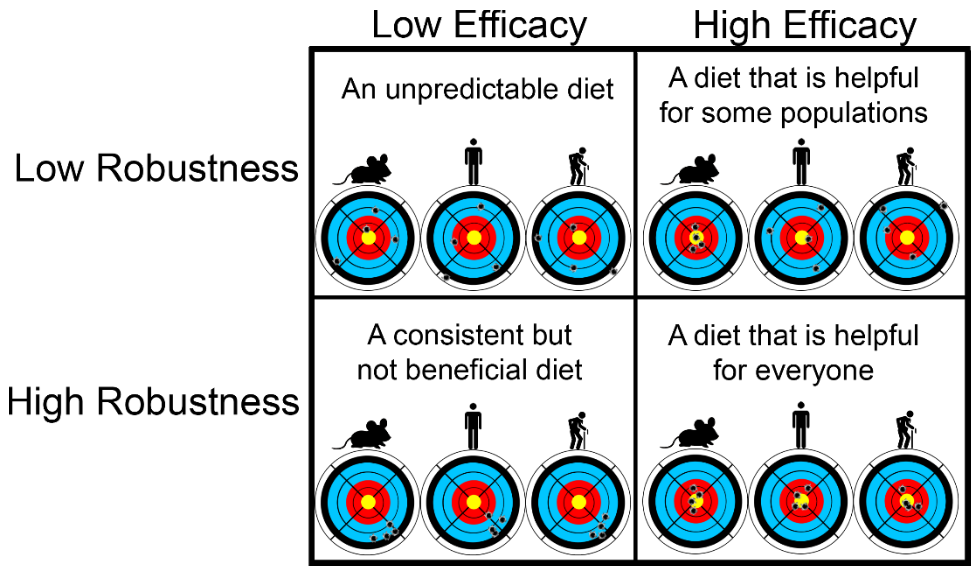
To understand this, we need to appreciate that we do not actually directly utilize the proteins we eat. Proteins are made of long strings of single unit molecules called *amino acids*. Our bodies break down proteins from foods into amino acids in order to rebuild them into the proteins that we need for our own biological functions. This is very much like repurposing a Lego set. An individual protein can often be thousands of amino acids long, and folds into complex structures to carry out its job. There are a total of 20 common amino acids, and humans lack the ability to produce 9 of them - these are the essential amino acids. We must either ingest them through our diet or obtain them from other sources to survive.



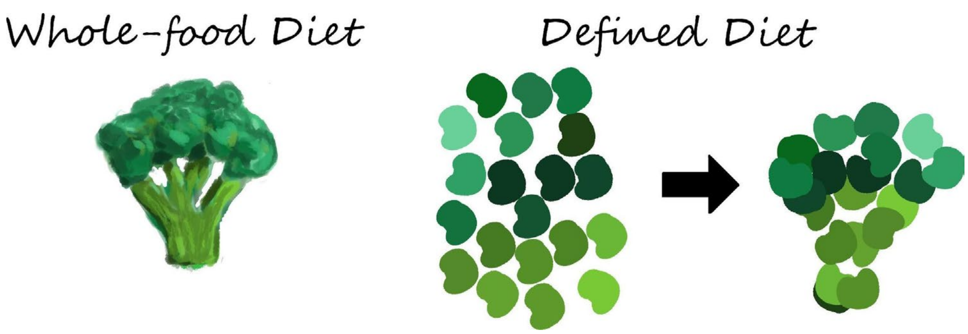
The most fascinating thing is that our body responds uniquely to different essential amino acids and this is the crux of several modern dietary research programs. The Lamming Lab is particularly interested in one subgroup of three essential amino acids called the branched chain amino acids: leucine, isoleucine, and valine. Previously, clinical data found branched chain amino acids to be persistently elevated in patients with diabetes, obesity, and other conditions involving insulin-resistance. We followed this up with a logical question: what would happen if we decreased the amounts of branched chain amino acids in the diet? To address this, we utilized mice as a mammalian model system, let them eat custom-made diets with various amounts of branched chain amino acids, and then examined the adaptations made by their metabolic systems using specialized tests. By using animal models in our dietary research, we can investigate and manipulate several variables in isolation to ensure that the effects we are seeing are real and not due to random variance.

Designing a research diet

I and others in the Lamming Lab meticulously design our diets and the experiments to ensure our scientific questions will be answered appropriately. We strive for reproducibility and accuracy in our results. Reproducibility means that if we or another laboratory repeats the same experiment, it should yield similar outcomes. A research finding is accurate if it correctly represents reality and answers the experimental question as intended. Similarly, our goal is to identify a diet that is both widely applicable and beneficial to everyone. The words that many scientists use to describe this is a treatment's *efficacy* and *robustness*. The efficacy of a treatment is its effectiveness in generating a desired effect while the robustness of a treatment is its consistency in generating that effect under various conditions. The higher a diet is in both efficacy and robustness, the more potential it has as a successful intervention. With these aims in mind, we scrutinize one thing in particular in our experiments - the composition of our test diets.



Reproducing dietary studies is notoriously difficult. As you can imagine, the same weight of grain harvested one year to the next year will have slight variations in nutritional content. Taking into account all the ingredients, these slight changes can combine to cause a significant, and unexpected, effect. To address this, we consistently order our diets from the same manufacturer, fully publish the ingredients of our diets, and use what we call the "amino acids-defined" diets. In these research diets, instead of proteins, we use amino acids. Essentially replicating the nutritional make-up of a diet using only its chemical units. You could say this is the opposite of a whole food diet and is something more similar to space food with all of the powdered nutrients pressed into feed pellets. While costly, this ensures that our diets can be easily reproduced at a later time or by another lab no matter the sources of their ingredients. Of course, this is not a realistic everyday diet, but for investigative purposes, this is the best approach.



Now to test our theories, we will add or remove different components of the diet. This is an extensive act of balancing between the macronutrients ratio and the caloric density, which is the calories per gram of food. In all of our experimental diets, we take special care to maintain these balances and eliminate unnecessary variables. In our example, we reduced the content of branched chain amino acids by 2/3 while increasing other non-essential amino acids to maintain the macronutrient ratio and the energy content. All other variables are kept the same. This way, the diet itself only has one significant change at a time, allowing us to attribute the results to our intended changes, which is the reduction of branched chain amino acids.

Dietary branched chain amino acids and the use of animal models

We performed several specific measurements on these mice fed the designer diet low in branched chain amino acids. For one, we used isolation chambers to measure oxygen consumption and carbon dioxide output of these animals. With a bit of calculation, we were able to put a number on the metabolic rate of these animals. Using other equipment, we tracked blood glucose levels, measured physical fitness, and performed memory tests. Our results demonstrated that by reducing the consumption of branched chain amino acids, mice became more metabolically active, ate more food but still gained less body weight, and had reduced body fat when compared to their companions that ate the regular diet. In addition, these animals had improved blood sugar control and, if they were fed the diet for life, had extended lifespan. In follow-up experiments, we further isolated the branched chain amino acids one by one. Out of leucine, valine, and isoleucine, we found that the reduction of isoleucine alone yields effects similar to the reduction of all three branched chain amino acids simultaneously. Surprisingly, reducing leucine or valine had very little effect, further highlighting the uniqueness of each essential amino acid.

The Lamming Lab uses various mice models in our dietary studies. Many model organisms are commonly used in research and they each are similar to the human body in their own ways. Examples include simple living systems, such as single cells in a petri dish, to more alien creatures like worms and flies, and more familiar mammals, including pigs and monkeys. Throughout my career, I have only worked with rodents – mice and rats, which are metabolically very similar to humans, but each model has their own strengths and weaknesses. As a category, animal models are indispensable in modern dietary research. Here are three key advantages:

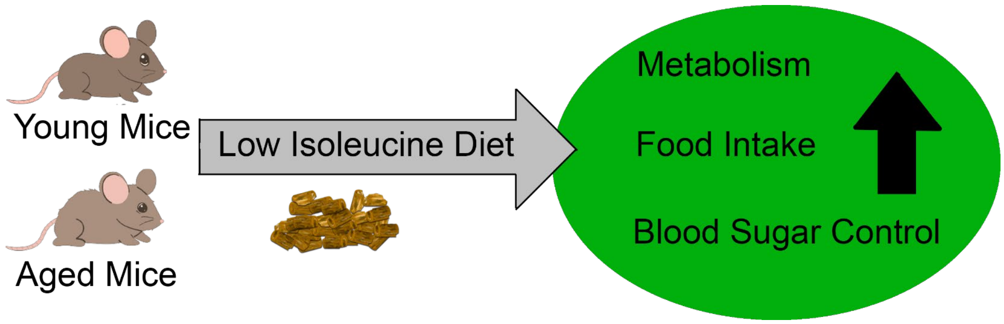
Animals adhere to the prescribed diet. Or rather, they do not have a choice. Unlike human studies, laboratory animal studies are highly controlled and we can more carefully document everything that the animal has access to. In human studies, where the participants have access to a vast variety of food, compliance is simply too poor and there are too many variables to account for. By using animals with 100% adherence to the diet, we can gain very reliable insight on exactly how one diet affects biological processes.

Lifelong studies. Laboratory animals do not live as long as humans and we can observe their entire lifespan while eating a single diet, taking note of their aging process and collecting valuable longitudinal data. In fact, we and other research groups have found several experimental diets to extend lifespan in mice and other animals. While it is unrealistic to eat the same food your entire life, these studies are important first steps to understand what is necessary to achieve healthy aging.

Testing diets in disease models. Animal studies are crucial steps leading up to clinical trials that may one day develop life-saving therapies. By using model organisms designed to mimic certain disease conditions, we can test the effect of a diet on these illnesses before testing it in humans. Some diets have been shown to be able to provide relief for several diseases, including Alzheimer’s disease, diabetes, and fatty liver disease.

My findings in aged mice

I wanted to continue the line of investigation on dietary isoleucine by taking advantage of the short lifespan of mice (2-3 years on average). In particular, I asked the question whether the benefits of reducing dietary isoleucine would remain effective when initiated in geriatric animals. In clinical practice, old age is an overwhelming risk factor for developing health complications. For practical reasons, any kind of health-promoting therapy would be infinitely more impactful if they remain beneficial in older adults. To simulate this, mice were naturally aged to 20-months-old, and I placed them on a diet with 2/3 reduction in isoleucine and observed the results over several months. At 20-months-old, a mouse is at the equivalent of a human at 60 years of age. My results found that the low isoleucine diet induced fat loss, lowered blood sugar levels, and increased metabolic rate. These animals eating the specialty diet became leaner and showed signs of youthfulness in their heart and liver. My findings support the idea that dietary intervention can be tremendously effective even at an advanced age. In addition, these animals lost a great amount of body weight. This may come as a surprise to you because these mice are not eating less food (nor are they exercising). On the contrary, they are eating more food and are, at the same time, losing weight. This contradicts the common idiom that “a calorie is a calorie”. In fact, the findings of the Lamming Lab as a whole suggest that the quality, and not just the quantity, of your diet is tremendously important for achieving healthy metabolism.



My current study in the Western diet

I began my career in research with the lofty goal of one day improving human health and thereby increasing the length of disease free human life. While mice are not simply small fuzzy humans, they are susceptible to similar diseases. For example, when fed a high-fat high-sugar Western diet, mice will also become obese and present diabetes-like symptoms. In my recent studies, I sought to determine how the Western diet changes the effects of reducing dietary isoleucine on a commonly used immunosuppressant with metabolic side effects called rapamycin (also known as Sirolimus or Rapamune). Rapamycin is commonly prescribed as an anti-rejection medication for organ transplants. This drug, first discovered in the soils of Easter Island, has been a cornerstone of aging research due to its ability to extend lifespan. Unfortunately, rapamycin induces a plethora of side effects, including the disruption of glucose homeostasis. In this study, I fed mice a Western diet for 3 months. When the mice reached 40% body fat, I switched their diet to a Western diet with reduced isoleucine content. Over a period of 3 weeks, their body fat percentage on average was reduced to 13%, along with a 44% reduction in body weight. During this period, I discovered that the metabolic side effect of rapamycin was mostly restored by the reduction of isoleucine in the Western diet.

This is an exciting and an intriguing result for me. I have previously performed this experiment without the use of a Western diet, and not only did the rapamycin side effect persist, but the drug actually blocked other benefits of the low isoleucine diet. Together, these data suggest that the dietary context (Normal diet vs Western diet) is important for determining the effects of the diet-drug interaction between a low isoleucine diet and rapamycin. I am currently working on further understanding how this is possible at the molecular level. Understanding this type of diet-drug interaction is very important from the perspective of patient care, and the eventual translation of our dietary interventions.

My future study in Alzheimer's disease mice

As a postdoc, I have my sights on finding my own niche in research and becoming an independent investigator. With the world's population rapidly aging, I am interested in identifying better ways to combat age-related diseases. One of the most pertinent and debilitating illnesses is Alzheimer's disease. From the diet front, we have shown experimentally that, in familial Alzheimer's disease mice model, a diet low in total protein started early enough can prevent the symptom progression of dementia. However, I have noted that these findings are inconsistent with a recent Alzheimer's disease patients' study, which found higher dietary protein consumption to be associated with reduced accumulation of disease biomarkers in the brain. One possible explanation for this discrepancy may be that different levels of dietary protein are optimal at different stages of the disease progression. This highlights the need for a more comprehensive study on how dietary nutrients interact with Alzheimer's disease development. To begin addressing this, I have submitted a proposal to the National Institutes of Health (the national funding agency for research), to simultaneously study the effects of low and high protein diets in genetically engineered mice with a propensity to develop Alzheimer's disease at different rates. If awarded, I will carry out this research in my own lab in the future. While this is years away, I am excited to contribute to our understanding of mental health during aging and to advance our understanding of dementia disease treatment.

The translation of dietary research – what does it mean for me?

I have always had the urge to eat like my healthier mice (in fact, many scientists do). However, the reality is that it is premature to be pasting our study results onto the cereal box, as we do not quite understand how humans would respond to these diets in the long-term. To reiterate our findings, the reduction of certain dietary proteins, especially isoleucine, induces an increase in metabolic rate, leading to weight loss despite no decreases in overall caloric consumption. This is very counterintuitive. The common assertion is that dietary protein is good for you and the more total calories you eat the more weight you will gain. While this is indeed the case for some diets, we have shown that there are many exceptions. This puzzling paradox is why we are proceeding cautiously with testing in people and giving dietary recommendations.

Scientific translation means to bring what we learned in research in a beneficial manner to the bedside – or to the kitchen table in our example. From the academic perspective, these animal studies build our understanding of how diets affect metabolism, which will eventually allow us to design appropriate clinical trials with an experimental diet or with a diet-imitating drug. Thoroughly carried out human trials can take years, if not decades. Why is this level of precaution necessary? The reality is that animal experiments are exceptionally good at answering precise questions, but in the process, they fail to account for all the complexities of daily life. Every day your activities are different, and that can change the responses to these treatments. Simply put, there is still a lot we do not know and it would be reckless to prescribe experimental findings to people too soon.

Nevertheless, quite a few correlational human data in small populations and short trials appear to support our findings in the animals. For example, in a survey of Wisconsin residents, we were able to find a strong positive correlation of dietary isoleucine consumption with Body Mass Index, more so than the other branched chain amino acids. In a short-term interventional study, a diet low in total protein, including a decrease in branched chain amino acids, successfully improved metabolic health by reducing blood glucose levels and inducing weight loss. However, these studies still do not fully capture the picture of everyday life and they reject the common assertion that higher protein intake is healthier. For these reasons, more cautious long-term studies are necessary before any official recommendations can be given.

Final Takeaways

Dietary research is one of the prominent topics in modern medicine and has great potential in treating many illnesses and improving the general health of the population. Before clinical trials, we utilize animal models, including mice, to test the effects of these treatments and to understand how our bodies could respond. A key aspect of dietary research is to appropriately design the experimental diets so that only one component is being tested at a time. Several labs have independently generated results that support the idea that decreasing the consumption of certain proteins may be beneficial, but long-term, well-controlled human clinical trials have yet to occur.