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Controlling the Controls

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Controlling the Controls

Arianna Schabauer with Dr. Donal Skinner

Senior Honors Project Capstone

Introduction

Scientists across the globe use other researchers’ work in order to base their own projects. Many of the experiments that could someday lead to new medicines and treatments for humans involve animals. Model organisms provide an avenue to study health and physiology that would otherwise be impossible in humans. These animals are highly standardized across most laboratories, however most research labs do not have standardized diets that are the same as other labs. These diets in all labs are called and considered ‘control diets’, however they are made up of differing amounts of macronutrients and micronutrients. Diets are often not listed and the ingredients not detailed in research paper methods, rather the research paper will cite that a ‘control’ diet was used. As will be shown in this senior research project, differing amounts of some of these ingredients, even to a very small degree, can have a very large effect on the overall health and body composition of the animal. This issue needs to be addressed by the scientific community, as this would allow research discoveries to be more significant, accurate, and efficient.

I have spent quite a large amount of time in classes and in Dr. Skinner’s research lab at the University of Wyoming doing research. These experiences had lead me very early on to the observation of this lack of reporting or matching diets in other research. After further research, this problem was glaringly obvious. Without the ability to find the composition of a cited diet, many resources could not be used in my papers. When considering the many topics possible for
writing this senior research project, the final decision came down to what was viewed as most
critical to change in the research world. The knowledge of this topic from this research,
combined with the knowledge gained in obtaining a BS in molecular biology (involving body
physiology and molecular mechanisms) and work in Dr. Skinner’s physiology research lab is the
fuel behind a good argument on the topic. I hope this paper is sufficient to convince the reader
of the changes that need to be made in the information reported in scientific papers.

Controls

It may be helpful to begin by defining a few terms. A ‘control experiment’ is a test where
the researcher conducting the test only changes one variable and everything else is treated
exactly the same in order to isolate the results and see exactly what effect that one change would
have. This experiment could be concerning any topic and the same basic control ideas and
definitions would apply. A ‘control group’ in an experiment is the group of test subjects left
untreated or unexposed to some procedure, and then compared with treated or exposed subjects
to validate the results of the test. This can be contrasted with an experimental group, which is
exposed to the variable and shows the effects, which are then compared back to the control group.
There are many controls that are important in research studies and particularly research studies
involving rats.

Controls in experiments, particularly experiments involving animals, are very
important to regulate. One example is the number of animals being housed together. Rats living
alone have different activity patterns due to the stimulation available. It has been shown that a
food-deprived animal would rather socialize when presented with company and food than eat
(Weiss et. al, 2017). This can be compared to other forms of stimulation that are placed in the
cage other than more animals, which will have the same distracting affect from the food. Another important example of a control is the age of the animal. As with humans and other animals, the body changes the most in the first few months of life and continues to change throughout its life. For this reason, a rat of a very young age should never be compared to a rat that is older because there are so many inherent variables. Another example is the size of the cage and the environmental stimulation found in it (as discussed a little before). A rat found in a smaller environment without an exercise wheel or things to interact with will perform much lower amount of physical activity than a rat with more space and stimulating objects. This speaks to the activity versus the food consumption of animals with environmental stimulation. A few more variables that must be controlled include temperature, lighting cycles, and water intake. The most relevant, and in my opinion important variable to keep constant, is diet. This is the variable that will be focused on for the rest of this research paper.

Diet is very important to control in an experiment, as diet directly affects the body physiology of the rat. Any change in diet from amount eaten to type of food eaten can have a huge effect. When performing an experiment, a control diet and an experimental diet should be properly reported and maintained. A ‘control diet’ is the diet the animals not being observed for changes will be fed. This diet should be tailored to the health of the animal in every way (with the exception to specifications of the experiment). The animals in the experiment being observed for changes are fed the experimental diet, which should be the same as the control diet aside from the single variable or group of variables being studied. The control groups in all experimental situations are important for isolating the results and seeing exactly what effect or effects that single variable would have.
In principle, to use a ‘control’ and compare one set of studies to another with the same ‘control’ is certainly legitimate, but to compare a ‘control’ to the results of another lab that used a different ‘control’ is illegitimate. This is precisely what is happening in research to this day. Labs are basing experimental results on several other labs’ results that supposedly did the same experiment, but the baseline diets were different. The experiment’s outcome very well may have been due to the intentionally altered part of the diet or of the experiment, but it may have also been due to the difference(s) in the ‘control’ diets. Conclusions cannot be drawn from these results, as there is too much variability and uncertainty, yet conclusions are being drawn leading to false results going unnoticed.

Another term that would be helpful to define is a ‘matched diet’. A matched diet is a diet in which from lab to lab is exactly the same in the ingredients. If all labs were to use ‘matched diets’ as their controls, the experiments would be able to be compared, and a result found. Many companies who manufacture diets do not even use the same units when reporting amounts of ingredients. It would not be hard for one to convert the units when using different diets, but it would be much easier if manufacturing companies would use the same units when reporting ingredients. Even with the fact that researchers can convert the units, when the ingredients are not even reported or the diet is not named in the paper, it is impossible to do this. Using the same control diet would be a much easier way to fix this problem.
Core Problem

Not controlling any one of the prior listed variables or others not listed may completely change the way one study compares to another. To think about this, imagine a 75 year-old man being compared to a 25 year old man in a study concerning fitness. The two men would be impossible to compare, as their entire physiology, lifestyle, and mentality are different. A very large source for error lies in the amount and type of food and nutrients being consumed by an animal. This is where a controlled diet would be important to minimize these differences.

From the scientific method, there are three baseline questions that should be able to be answered in every experiment. These are “Can I report it?”, “Can I repeat it?”, and “Can I revise it?” (Ricci, 2013). If a diet is not properly matched in an experiment or is not included in the details of the research paper, that research cannot be reported. If the diet is not matched properly or included in the paper, that experiment cannot be compared or repeated by another research lab or group. If this group is not able to properly repeat the experiment, the method cannot be revised. The core problem the scientific community must address is the diets not being matched and published, and these three questions being answered with ‘yes’, when they should be answered with ‘no’. Sometimes taking a step back to the basics of proper research methods can allow researchers to fix simple problems that they were not aware of.

Diets

There are two types of diets that are important to define for the rest of the paper. These are the grain-based diet and the purified ingredient diet. These two diets should not be compared, as they have very different compositions of nutrients (Ricci, 2013). Grain based diets contain some heavy metals which can be toxic to rats and other animals, as well as phytoestrogens.
Phytoestrogens are xenoestrogens, which are not made in the body and are taken into the body when consuming plants. Phytoestrogens can have large effects on sexual maturity, cancer, and bone metabolism of rats (Odum et. al, 2001). Figure 1 illustrates the amounts of two types of photoestrogens (Daidzein and Genistein) in 5 different control diets used in rat experiments. AIN-76A was state in the research paper as having no phytoestrogens.

![Figure 1: Amount of Phytoestrogens in Control Diets](image)

As can be seen, there were very different phytoestrogen content in each of these supposed “control” diets. The study compared organ weight in groups of rats fed these different control diets to see the effects of the phytoestrogens on the body. These organ weights were taken from
euthanized rats at specific time intervals throughout the study. After statistical analysis, the significant results of this experiment can be seen in Table 1. Purina 5001, which you can see in Figure 1 had the highest amount of phytoestrogens, was used as the control in comparing and reporting the results.

Table 1: Significant Changes on Post Natal Day 68 with Purina 5001 as Control

<table>
<thead>
<tr>
<th></th>
<th>AIN-76A</th>
<th>RMS</th>
<th>Global</th>
<th>RM3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>Decreased Size</td>
<td></td>
<td>Decreased Size</td>
<td>Decreased Size</td>
</tr>
<tr>
<td>Liver</td>
<td>Increased Size</td>
<td>Increased Size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry Uterus</td>
<td>Decreased Size</td>
<td></td>
<td>Decreased Size</td>
<td>Decreased Size</td>
</tr>
<tr>
<td>Testes</td>
<td>Decreased Size</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As can be seen, AIN-76A had the most significant changes in organ weight. This would make sense as AIN-76A was said to have no phytoestrogens and was compared to the control diet containing the most (Odum et. al, 2001).

In multiple experiments, grain based diets are used as the control diets while purified ingredient diets are used as the experimental diets. Purified ingredient diets do not contain these phytoestrogens and therefore do not have this variability. If these diets were compared to one another, the results of the experiment could be due to the phytoestrogens, and not the nutrient being studied. It would be difficult to conclude the experimental variable was the reason for a physiological change when there were so many other variables just between the diets. So, results should not be reported when the control and the experimental diets were not properly matched.
Also important to note is that each of the five diets in that study were considered “control” diets and used as such in experiments. When the control diet is not cited in a paper, or is just simply called a “chow”, too much variability exists right there for a reviewer to move forward with repeating the experiment or comparing results.

Dr. Skinner’s Lab

It is a generally accepted result in the rat research world that a high fat diet will advance puberty in young rats. The day of vaginal opening for female rats is considered their first day of puberty. When being studied, vaginal opening is checked every day or several times a day, and samples taken of the cells in the vaginal opening when needed for the experiment. One example of research, which found this advancement of puberty with a high fat diet, can be found in Figure 2 (Wirasak, 2012). Female rats were fed either a high fat or no fat diet and checked for vaginal opening.

Figure 2: Day of Vaginal Opening Between High Fat and No Fat Diets
As indicated in the figure, the high fat diet rats began to start puberty 6 whole days before the non fat diet-fed rats (Wirasak, 2012). Contrary to these results and many other similar results, personal research in Dr. Skinner’s lab found quite the opposite result. Figure 3 shows the day of pubertal onset of rats in Dr. Skinner’s lab fed either a high fat or a low fat diet.

Figure 3: Day of Pubertal Onset Between Rats Fed High or Low Fat Diet

Interestingly, no change was seen between the high fat and low fat fed rats in Dr. Skinner’s lab, which was opposite of the results seen in previous studies. This brought up many questions as to why this may be occurring. Sifting through possibilities and ideas, a hypothesis
was made. There is a window in which puberty begins for female rats. Perhaps the control diet being used in the studies in Dr. Skinner’s research was already pushing puberty as early as possible in that pubertal-onset window. This lead me to the research question, what about the controls?

Most diets considered control diets for rat research have very different amounts of nutrients. After doing some research on the subject, four of the main control diets used are listed in Table 2.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>SDS</th>
<th>LabDiet</th>
<th>Teklad</th>
<th>Open Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar</td>
<td>4.05</td>
<td>3.45</td>
<td>n.r.</td>
<td>n.r.</td>
</tr>
<tr>
<td>Starch</td>
<td>44.97</td>
<td>28.6</td>
<td>n.r.</td>
<td>n.r.</td>
</tr>
<tr>
<td>Fat</td>
<td>n.r.</td>
<td>11.4</td>
<td>4.7</td>
<td>10.5</td>
</tr>
<tr>
<td>Protein</td>
<td>14.38</td>
<td>23.2</td>
<td>24.3</td>
<td>16.4</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>.63</td>
<td>.79</td>
<td>n.r.</td>
<td>n.r.</td>
</tr>
<tr>
<td>Calcium</td>
<td>.73</td>
<td>.95</td>
<td>n.r.</td>
<td>n.r.</td>
</tr>
<tr>
<td>Potassium</td>
<td>.67</td>
<td>1.15</td>
<td>n.r.</td>
<td>n.r.</td>
</tr>
</tbody>
</table>

As can be seen, each diet differs in amount of most of the nutrients reported on the information sheets. Next, the affects these differing nutrients can have on the animal and research will be discussed.
**Macro and Micro Nutrients**

The ingredients making up the diets in question can be broken down into macronutrients and micronutrients. Macronutrients are the major components of diets that are consumed, and they constitute the bulk of our caloric intake. Varying the ratio of macronutrients to one another can have vast and drastic impacts on the physiology of the animal. Carbohydrates, protein, and fat are the three main macronutrients of importance that will be discussed in this research paper. These effects can be very significant or small, but all cases should be taken into account when reporting results. This result has been seen not only in papers found when conducting this research, but personal research in Dr. Skinner’s lab at the University of Wyoming. Conclusions can be drawn from this research, as there are so many different examples to both a small and a large scale.

Carbohydrates consist of starches, sugars, and fiber. The process of glycolysis involves the breakdown of glucose into ATP to be used as an immediate source of energy throughout the body. In order for normal body functions to occur, a constant energy supply is important, so glucose can also be stored as glycogen. This glycogen can then later be broken down in the process of glycogenolysis. These processes are regulated by hormonal signals for high or low blood glucose (blood sugar). One of these important hormonal signals is insulin. Insulin is a hormone that is excreted from the pancreas when the amount of glucose found in the blood is too high. Insulin stimulates the cells to store more glycogen, which can then be used later. Another important hormone in the body is glucagon. In opposition to insulin, glucagon is a hormone that which is excreted from the pancreas when the amount of glucose in the blood is too low. Glucagon will promote glycogenolysis when present, which will raise the amount of glucose in the blood.
The first type of carbohydrate to be discussed is starch. Starch is a fermentable carbohydrate that can significantly shift the absorbability of major cations in the body (especially calcium). Some examples of cations in the body include calcium, magnesium, and potassium. These are all heavily involved in many of the processes in the body. If the cations were not able to be absorbed, many body processes would not function properly. An experiment would be hard to carry out with accurate results if one organism had normal body functions while another rat was suffering from some internal issues due to a different diet. No conclusion in an experiment would be able to be made if this was the case. In simple terms, the more carbohydrates present, the more energy available for immediate use or storage. A higher amount of energy could lead to higher activity of the organism, which leads to changes in muscle composition, fat composition, and many other physiological components. With lower amounts of starch, less glucose from diet would be obtained therefore affecting the energy and activity of the organism. Here are some other examples of research, which have looked into the effect of varying amounts of starch in rat models.

Rats in a first example were fed either 54% starch or 54% sucrose diets. Several different feeding patterns were tested, but they did not have any significant differences. The differences came from the type of diet. The rats fed the high-starch diet showed higher insulin sensitivity, meaning a small spike in blood sugar caused insulin to take action. In contrast, the rats consuming the high-sucrose diet generally exhibited significantly higher fasting serum insulin levels than rats consuming the starch diet. Serum insulin levels are the amounts of insulin present in the serum of an animal, so fasting serum insulin levels would be the amount of insulin present in the serum of animal that is fasting. Since obesity, insulin sensitivity, and hyperinsulinism (high insulin level) are all associated with impairment of glucose tolerance, the
observed metabolic effects of dietary sucrose are considered to be undesirable as compared to starch (Reiser, S. and Hallfrisch J., 1977).

Sugar is a short chain soluble carbohydrate, which is broken down in the body as glucose to be used in glycolysis to be turned into energy. Sugar is a fermentable carbohydrate, which means it is made up of a short chain that is easy to break down in the body. Sugar is broken down and turned into ATP, which is the body’s main source of energy that is utilized to continue normal bodily functions. Monosaccharides such as glucose, fructose, and galactose can be broken down to make ATP through different pathways. Diasaccharides are similar to monosaccharides except they are in essence two molecules instead of one. Examples of these are sucrose, maltose, and lactose, with the most common in food being sucrose. Though all are slightly different in the body, the main goal is to break these down for energy for the organism. Next are some examples of effects that can occur when sugar diets vary.

In an experiment done on rats to test sugar dependence, rats were fed excess sugar intermittently to see the effects of behavior and intake. All of the rats were then put through a fasting period and then given the option of consuming sugar again. The rats fed more sugar compared to the control animals showed a higher intake of sugar than ever before, leading the researches to believe these rats were sugar-dependent under selected dietary circumstances. This sugar dependency also lead to some behavioral and activity-level changes in the animals. Another result stated a rat with a sugar-dependency will eat more than a rat that is not, which could create a significant difference in the amount of body fat and other major body compositions. As in humans, rats that are heavier will exercise less and their major organs will quickly become affected. These body and behavioral changes in the rats would be a major variable in an experiment (Avena et. al, 2005).
Another example of how a difference in sugar can affect the body involves rats eating the same amount of food made up of different percentages of sugar and fat. The rats eating the sugar-rich diet became severely obese in the absence of hyperphagia, even though they were eating and excreting the same amount of food. Hyperphagia is defined as abnormally increased appetite for consumption of food frequently associated with injury to the hypothalamus. So in other words, the animals that are eating a lot of sugar, despite the fact that hyperphagia is not involved, can become obese. A severely obese rat or even a rat that is heavier than another has very different body composition from a normal BMI rat. Bodily functions, exercise/activity levels, and behavior can all be very different. This would be a similar comparison to a morbidly obese or heavier human being compared to an average, active human. Similar to the last example, it is clear how an obese rat in an experiment could show different results in an experiment than a rat of normal weight and would skew the results (Oscai et. al, 1987).

Dietary fiber, or roughage, is the indigestible portion of food derived from plants. Dietary fibers can act by changing the nature of the contents of the gastrointestinal tract, and by changing how other nutrients and chemicals are absorbed. Some types of soluble fiber absorb water to become a gelatinous, viscous substance, which is fermented by bacteria in the digestive tract. Fiber can help to slow the breakdown of other carbohydrates, which helps with blood sugar control, the cardiovascular system deliver blood to other organs, weight control, and the gallbladder, kidney, and bowels (Mayo Clinic Staff, 2015).

Beta-glucoronidase and mucinase are families of enzymes that break down complex carbohydrates in the body. In an experiment, the specific activity and total output of beta-glucuronidase were highest in rats fed fiber-free diet and significantly lower in those fed 15% fiber diets. A higher output of beta-glucuronidase means there is less carbohydrate breakdown
occurring within the body or an overproduction of glucuronidase—either way the body is functioning slightly differently to break down carbohydrates. This could mean more or less storage of glucose in the body and slower response to meals. Some kinds of dietary fiber may also play a role in the etiology of intestinal disease. A rat that is more prone to intestinal disease may not be the best candidate for an experiment due to the obvious variables that could create differences in the experimental results (Shiau, S. and Chang W., 1983).

In another example, two groups of diabetic rats were given either brown rice-fiber or soybean fiber to test cholesterol levels, plasma insulin and glucagon levels, and glucose tolerance tests. The results showed that the diabetic rats consuming the soybean fiber had a significantly lower serum glucose levels than the control diabetic rats not consuming the fiber. Soybean fiber also significantly lowered the glucagon and triglyceride levels in both control and diabetic rats. Comparatively, rice fiber had no effect on the rats on these blood parameters. Glucagon signals for low blood sugar, so significantly lowered glucagon levels would indicate higher blood glucose levels or perhaps impaired ability of the body to signal for low blood sugar as efficiently and affectively. Any experiment with this many variables would not be considered valid in the scientific community (Madar, Z., 1983).

Unlike carbohydrates, fats are much more calorically dense. They can be broken down into a glucose derivative, which is then used for energy. Fats are constructed as a triglyceride, made up of three fatty acid tails with a glycerol backbone. Some fats are considered healthier than others due to the way the body responds to and uses them. Saturated fats, found in animal products such as butter and lard, are fats that are completely saturated with hydrogen atoms and therefore are completely straight molecules. Saturated fats also have no double bonds between the molecules. The saturated fat molecules pack together much more efficiently than molecules
with a bend, like unsaturated fats. Unsaturated fats can include any number of double bonds from a single double bond to many. An unsaturated fat containing a single double bond is called a monounsaturated fat; any more than a single double bond in a fat is called a polyunsaturated fat. These fats can be found in sources such as plants and nuts, and oils made from these sources. There are very large differences in the chemistry of saturated versus unsaturated fats, and there have been many experiments done to investigate the health benefits of both kinds. The body physiology in rats, which can be altered due to both kinds of fats will be investigated here.

A carcinogenic chemical is thought to cause or be associated with causing cancer. A neurotoxic chemical is one that is proven to cause damage to nerve tissue. Azoxymethane is a carcinogenic and neurotoxic chemical compound used in research to induce colon cancer. In this experiment, rats were treated with Azoxymethane and fed either a saturated fat diet or an unsaturated fat diet to compare the severity and rate of progression of the cancer. The rats fed the saturated fat diet had less severe progression of the cancer as well as a much smaller number of colon tumors from the cancer. It was concluded the unsaturated fat diet also made the rats more susceptible to the chemicals, which induce the colon cancer. With such drastic results on how cancer could progress in rats, feeding diets which may vary in the amount and type of fat composition in an experiment could lead to major variables (Sakaguchi, M. et. al, 1984).

In another experiment, sugar and fat were examined in animals to see the differences in body and behavior. Addictive-like behaviors were found in animals on sugar and fat binges. A more obvious result was found as a high-fat diet seemed to be the major reason for weight-gain in the animals. High-fat was also found to be the major reason for additive-like behaviors when it comes to food. The research found that even small changes in the amount of fat present in a diet has been shown to affect an animal in a way that changes how other nutrients can be absorbed.
Not only does this then affect the weight/obesity on an animal, but also how healthy they are in many other physiological functions. It would be close to impossible to achieve accurate, definitive results with so many variables (Avena, N. et. al, 2009).

Continuing with the idea of obesity connected to high-fat diets, some researchers hoped to find a rat model, which was similar to the metabolic disorders found in humans. This experiment aimed to find which types of fats would generate these disorders in the rats which resembled the human metabolic disorders. The fish-oil fed animals did not develop the obese or insulin-resistant phenotypes, while the animal fat and omega-6/omega-9 plant oils did. Once again, obese and insulin-resistant rats will function differently. This experiment showed that perhaps the fish-oil is a more healthy fat than the plant or animal oils. Many of these different fats are contained in foods for all animals. If a certain fat which induces less obesity is found in one food used in an experiment with another type of fat being used in the other food, many phenotypic changes would be found. A good question brought on by this example is what type of fat then does create the ideal high-fat diet (Buettner, R. et. al, 2007).

In a final example of how fat can affect the physiology in rats, a dietary treatment using difference high-fat diets was fed to rats. At the end of the dietary treatment, the lipid mass of subcutaneous, mesenteric, retroperitoneal, and epididymal fat depots were measured. The total adiposity of control rats compared to those fed the high-fate diets of each fat depot was significantly lower compared to all of the three high fat groups. The lipid gain was due to fat cell hypertrophy (enlargement of organ due to the enlargement of the cells) and hyperplasia (enlargement of the organ due to increased rate of cell reproduction). This once again shows an increase in fat stored in an animal, affecting the animals’ physiology as well as their quality of life (Belzung, F. et. al, 1993).
So far, carbohydrates and fats have been discussed. The next macronutrient to be discussed is protein. Proteins are made up of amino acids, which are necessary in the body. These amino acids are used to build and repair tissue and muscles, make enzymes, hormones, and other chemicals, and are important building blocks of bones, muscles cartilage, skin, and blood (Effect of administration of fermented milk containing whey protein concentrate to rats). As you can tell from the previous description, proteins are important for the structure and function of many parts of the body. From this we can assume a change in the protein intake could affect a lot of things. This would be hard to keep track of in an experiment that was not properly using a protein control diet, or matching the experimental diets to the control diet.

Hypercalciuria is the condition of elevated calcium levels in urine. Another experiment proposes high protein feeding which causes hypercalciuria is due to the production and excretion of sulfate. This could mean the body is not absorbing calcium as it should, or that the body is dehydrated. The high protein diet would therefore affect how much calcium is being taken back into the body versus excreted out in urine. Calcium is very important for proper nerve muscle function, as well as many other things in the body. This can be a clear indication of the health and health problems an animal is experiencing as calcium is involved in many functions of the body and the cells in it, all due to high protein feeding (Whiting, S., Draper, H., 1980).

In interesting study to include studied the effects of baby rats when their mothers were fed low-protein diets while carrying them. This study shows not only the importance of control diets and matching diets during an experiment, but even in the lab while the experiment is still being planned. The major finding was that the blood pressure of the rats was very high compared to normal rats of the same age. Looking further into the body physiology, the researchers found renal structure, renal hormone action, and hypothalamic structure being altered compared to the
controls. Altered internal structures can definitely have large effects on animals as well as high blood pressure. High blood pressure can lead to damage to the heart or arteries or lead to heart disease and heart failure (Langley-Evans, S. et. al, 1996).

Uremia indicates the presence of urea (a major component of urine) in the blood. It can suggest an excess of amino acid and protein metabolism end products, such as urea and creatine in the blood, which would normally be excreted as urine. A moderately restricted protein diet securing only the minimal requirements had a beneficial effect on growth and survival of rats with reduced kidney mass. Avoiding any excess in proteins from the early stage of renal disease is suggested. This result seemed surprising, but nonetheless shows yet again another example of how changing the amount of protein does in fact have effects in the physiology of the rat (Kleinknecht, C. et. al, 1979).

Table salt (sodium chloride) is very important in our everyday diet. Sodium from salt is important for transmitting nerve impulses, muscle contractions, maintaining a proper fluid balance, and many other important bodily functions. From my time studying at the University of Wyoming, sodium has been one of the most studied and important micronutrients and electrolytes in the body. There is a reason salt has been an important and prized item historically, even being used for trade for goods. Not eating enough salt can lead to serious health issues including brittle bones, attention disorders, and muscle disorders. It has also been hypothesized that eating too much salt is bad for the body and can lead to excess weight gain. Either way, it is clear that changes in salt content in diet could lead to changes in health of a research animal.

Gastritis is a disease associated with irritation or inflammation in the stomach. Tumors and sores in the stomach can form due to the dangerously low pH associated with gastritis, which could lead to cancer. The *H. pylori* bacterium is strongly associated as being a cause of gastritis.
An experiment was conducted regarding this topic, to see if NaCl caused any changes in these rodents. This research found that excessive NaCl intake enhances the *H. pylori* in mice and in humans, so chronic salt intake may worsen gastritis by increasing their colonization. Gastritis is a fairly severe condition, and any animals with signs of this could have incredibly different anatomies and problems. Experiment where even significant changes in the stomach pH of the animal would alter how well and quickly food is being digested (Fox, J. et. al, 1999).

Osteomalacia is the misforming of bones. A lack of calcium in diets was shown to be a cause of this in another research article. Osteomalacia could lead to bone fractures and bowing. Rats with this condition might not be able to move as well or in the same way, and would not get as much exercise. A lack of exercise is a major cause of obesity and many other health problems (Harrison, M., Fraser, R., 1960).

Gentamicin is an antibiotic used to treat several types of bacterial infections. Nephrotoxicity is a poisonous effect of some substances, both toxic chemicals and medication, on the kidneys. Calcium is used in the body to reduce gentamicin binding to cell membranes. In an experiment, calcium loading was tested to see the effects of gentamicin nephrotoxicity. The results basically showed that calcium loading reduced the poisonous effects of the antibiotic gentamicin, which would negatively affect the organism as the organism would no longer be able to kill the bacteria using the antibiotic. Though gentamicin is not used in every experiment, it is important to note these results moving forward of high calcium in the diet (Bennett, W. et. al, 1982).

Another micronutrient, which is important to discuss is potassium. Potassium is an electrolyte as sodium and chloride are, which means it conducts electricity in the body in the form of nerve impulses. Potassium is important for skeletal and smooth muscle contraction,
which would include the heart muscle, digestive tract, and voluntary muscle movement. If an animal did not get enough potassium (hypokalemia), their activity level could be lower than normal, the heart function could be different, and the way food is digested could be different. All of these changes would be important to keep controlled in an experiment, so using a diet with controlled levels of potassium and other electrolytes would be very important.

Amongst other important functions, the kidney is the organ, which filters the blood and maintains electrolyte balance throughout the body, including potassium. An experiment was done to see the changes in the kidney based on potassium consumption. Results from this experiment showed depleting potassium lead to early increase in formation of cell membrane phospholipids. Early formation of phospholipids would mean the cells are not able to form and carry out their life cycles correctly. A change in this organ could then lead to big changes in the rest of the body, where the blood and nutrients are delivered. (Toback, F. et. al, 1976).

**Answering the Question**

Referring back to the confusing results as stated before, Dr. Skinner’s research lab aimed to find support for the hypothesis that puberty was occurring earlier when using Research Diets control. As a reminder, high fat diets advancing puberty in rats was a commonly found result in many research labs, but Dr. Skinner’s results in the lab found the opposite of that. The hypothesis was that perhaps the control diet was already pushing puberty as early as possible in the female rat’s puberty window, where a high fat diet would not be able to push puberty any earlier. To test this theory, two groups of rats were fed two different control diets. The control diet being used in Dr. Skinner’s lab already (Research Diets Control), and one being used in a
lab who were consistently getting the advanced puberty result (RM1 Control). The results of this experiment can be found in Figure 5 below.

Figure 5: Vaginal Opening in Rats Fed Different Control Diets

The results of this experiment supported our hypothesis. The Research Diets Control that was being used in our studies of high fat diets and puberty were advancing day of pubertal onset significantly compared to the RM1 diet being used in another lab getting the advancement of puberty. This shows a clear example of how two labs doing the same experiment with different controls skews the results. It is also important to note that the RM1 diet is not available in the United States, and a member of Dr. Skinner’s lab had to travel to England to pick up this diet.
This will be discussed again later, but this could lead to even more variability when the same diet cannot even be attained. Possible reasons for these differences could be the phytoestrogen contents, or the nutrient content.

**Other Perspectives**

An interesting perspective that could be worth mentioning is that of a known rat lover. This person does not perform experiments with rats, but has stated what she considers to be a healthy diet for rats. Debbie “The Rat Lady” Ducommun is famous for her love of rats online. She explains in one source on her website, the ingredients of a diet she uses for her beloved pet rats. She suggests twenty percent protein, eight percent fat, and three percent fiber. She says more than twenty three percent is bad for the rat’s body. Debbie has been a pet lover for decades and has studied rat health and tested drugs to treat rat disorders. Debbie is a famous advocate for rats, and wants to feed them the best nutrients they could possibly get. When looking back at Table 2, this diet is most similar to the LabDiet control, but not exactly the same. Maybe there is something behind her theory, and perhaps that could be investigated in another research experiment.

Another interesting perspective to discuss in this paper is what rats will eat given the choice. If rats are given a control diet with set percentages of fat, protein, carbohydrates, and various micronutrients, the only variable can be how much the rat eats. In contrast, in the wild the rats are given the choice of what ratio of these nutrients they wish to consume, based on what they feel their bodies need. Experiments hoping to learn more about this have been conducted. The select-reject decision process involves careful evaluation of the characteristics of the food such as flavor. With experience, animals refine their choices as they associate the flavors of specific foods with the foods have on their bodies. It is now well documented that strong food
preferences can also be learned as animals experience positive effects from the food (Sclafani, 1995). This could be a way to gain valuable information, as rats in the wild will eat upon need and raw instinct.

Conclusions

A thought that has probably come across your mind as it did mine, was how this research can be applied to humans. The phrase has been used a lot when talking about health of people: you are what you eat. Physiology can be significantly changed in humans as with rats due to diet. Obesity, heart disease, diabetes, etc. can all occur based on diet. Finding the perfectly healthy diet for people might not be as simple as it is for rats. It is important to mention how rats and humans eat very differently because the body physiology is very different. The results of all experiments with rats cannot be directly compared. Many effects discussed can apply to people, but many cannot. Though this is perhaps more complicated than with rats, we might actually have more knowledge about humans than rats. A control diet for all experiments with people would not be possible, as there are so many variables with all of the experiments being done. The results in these experiments are also easier to determine as people can directly answer questions such as, “How do you feel?” and “Are you hungry?”.

More important considerations can now be made between grain-based and purified ingredient diets. Arsenic (a toxic heavy metal) in diets can affect tissue gene expression. Having these chemicals in one diet and not another can leave a lot of room for variability. A purified ingredient diet contains about five percent fiber, while a grain-based diet contains twenty to twenty five percent (Ricci, 2013). As discussed earlier, fiber can have a large effect on body physiology in a rat. Soluble fiber is fermented by gut bacteria and has significant effects on gut morphology, inflammation, and microbe populations. Looking at Debbie’s suggestion, she
suggests a fiber percentage much closer to the purified ingredient diet. This may need to be investigated further, but perhaps could be a more accurate percentage.

Mismatching diets is very common in two ways. Experimental diets not being matched to the control diet is happening surprisingly often, even though it may seem like a no-brainer. The results of these studies as stated before may be due to the variable being tested, but could also be due to the phytoestrogens, toxic heavy metals, or differences between nutrients between the two diets. The significance of these differences was highlighted in all of the research of the effects on the rat’s bodies between different amounts. Diets are also being mismatched when experiments are being compared or repeated between several labs. This was very apparent in the experiment where one lab found a very significant advancement of puberty with a high fat diet, while Dr. Skinner’s lab found no difference. These results would not be able to be compared or considered valid.

Based on the scientific method, there are 3 baseline questions which must be answered in every experiment. These are, “Can I report it?”, “Can I repeat it?”, and “Can I revise it?”. In the advancement of puberty due to high fat experiment, the control diet and contents were properly reported, the experimental (high fat) diet was properly matched and reported, so “Can I report it?” could be answered ‘yes’. However, the control diet being used was not available in the United States, so the answer to “Can I repeat it?” would be no. The fact that mismatched diet studies are published in popular scientific journals and not criticized suggests that neither the authors nor the reviewers are aware of the problems inherent in comparing data from groups of animals fed completely different diets.

This leads to another important point of this research project. Perhaps the answer to this variability occurring in so many rat research experiments and probably all areas of research
involving diets and animals, would be introducing a worldwide control diet. A worldwide control diet could be created for each breed of animal and type of study, so that all research could be standardized. Experiments would need to be done on all types of animals and experiments in order to find a definitive answer for the best of each type of nutrient. Though this may take a while to complete, the efficiency and accuracy of all following experiments would be astronomical. Comparisons would be able to be made quicker and easier, and conclusions could be drawn more quickly. Fewer experiments would need to be done, as well as fewer experimental animals needed. Science as a whole could evolve in a positive way due to this change.

Thirty four percent of the time there is insufficient data about the diets in the methods section of a research paper (Ricci, 2013). This means, describing diets in detail was not considered important by the authors or reviewers thirty four percent of the time! As the reader may realize how important this information is now, this is a strikingly and scarilly high percentage. That means that perhaps thirty four percent of all research studies which controlled diets and studied the effect of nutrients on the body may have found false results and made false claims. The simple task of reporting this information could have made the difference in these experiments. The time, money, and animals used could have been saved for a project that came to valid conclusions. The other fact to think about is most reviewers of this material are not aware of which of the research projects are drawing these false conclusions because it has not been noticed or changed. Research is done all of the time simply comparing the effects on animals when a diet is changed in order to learn from and hopefully utilize for human studies. Unknowingly, many other labs have been doing this research simply by not using matched diets from control diet to experimental diet.
All in all, it is incredibly important that all research should be using matched diets within the labs, and the same control diets when spread across different labs. When experimental animals are fed a special diet, the control animals should be fed a diet matched in every way to the special diet except the dietary variable that the research is studying (Ricci, 2013). Even if different diets were used, the results could be normalized so that they have the ability to be compared. This would be the case for research being done now, as a worldwide control is not available. This would somewhat compensate for the different makeups of the diets. Reducing variability means that all researchers will have greater capability to make a real difference, and show come to important research conclusions. Ultimately, fewer animals may be required in order to achieve the desired result (Ricci, 2013).

I hope this paper has served to convince you of the importance of addressing the issue of non-matched diets and ingredients not being reported in research papers. Non-matched diets within labs means the diet being used as a control is made up of difference ingredients than the experimental diet, and can lead to huge physiological changes. In order to have a proper experiment, the only variable should be the topic of the research question. It is very difficult to control every single variable in an experiment as some simply cannot be controlled, but those that can be are crucial to pay attention to, or to report so other labs can normalize and take those variables into account when trying to compare.

Another issue is labs comparing results with other labs that did similar experiments, without questioning which ‘control diet’ was used. Many examples of how changing different nutrients in a diet can affect the animal’s body physiology were discussed, and I hope it is clear how important it should be to minimize this variability. It would not be valid to compare two
labs results when there were so many variables between the two diets used in the labs. If one control diet for rats were used universally, the efficiency and validity of studies done on rat health would be enormous. This could then in some ways be more quickly turned into usable data for human health and wellness. Research could become revolutionized in a whole new way and could lead to conclusions that can only be imagined.
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