“Examining the Effects of Natural Dietary Supplements on Fat Metabolism in the Fruit Fly, *Drosophila melanogaster*”

An Honors Thesis

by

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2017

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Acknowledgements

I would like to thank my advisor and mentor Dr. Louise Nicholson, PhD for her consistent support throughout the entire research process. Without her guidance, my research idea would not have transpired into the thesis project I am so proud to present. Thank you to the Center for Undergraduate Research at California University of Pennsylvania for funding this project. Without this funding, the research would not have been possible.

Additionally, I want to thank my thesis committee who helped read and fully develop the written portion of my thesis. My thesis committee consists of Dr. Louise Nicholson as my advisor, Dr. Chadwick Hanna as my second reader, and Dr. Gregg Gould as my Honors Advisory Board member, Loring Prest as my librarian, along with my Honors Thesis professor, Dr. Craig Fox, and Honors Program Director, Dr. Mark Aune.

Abstract

The increase in sedentary lifestyles and calorie-dense diets have made obesity prevalent in our society leading to a rise in obesity-related health problems, including heart disease and diabetes. While some prescription drugs are effective in controlling weight, patients are often concerned about adverse side effects. This has led to celebrities and popular media endorsing the use of natural dietary supplements, such as plant extracts, to combat obesity. The fruit fly, *Drosophila melanogaster*, can be used as a model to study obesity. This is because many genes and pathways involved in fat metabolism are shared between humans and flies, including the adipokinetic hormone (AKH) and mir-14 genes, which up- and down-regulate fat metabolism, respectively. This project's goal was to determine if four natural supplements – *Garcina cambogia*, raspberry ketones, cayenne, and vitamin B12 – effectively increase fat metabolism. These were tested on wild-type flies and flies with mutations in the *Drosophila* AKH and mir-14 genes. Sixteen types of fly food were made, each containing one of the supplements, with four different doses tested for each. Concentrations were based on recommended human doses. Wild-type flies were kept on supplemented and unsupplemented food for one week. Fat stores were then assayed using a colored colorimetric assay to measure triglyceride levels. It was found that cayenne at the highest dose significantly decreased triglyceride levels in male wild-type flies. Cayenne also caused a significant increase in triglyceride levels in the dAKH male mutants at the lowest dose, suggesting that cayenne's effects may be mediated in part by the dAKH pathway.

Introduction

Obesity and Its Effects

Obesity has become a burden to health care in the last few decades due to the growing number of associated health complications. According to the Center for Disease Control and Prevention, in 2013, 37.9% of Americans aged 20 years and older were obese and 32.8% were overweight (1). The average weight of a woman today is 166.2 pounds which was the average weight of a man in the 1960s and the average weight of a man today is 195.5 pounds (2). While the prevalence of obesity has risen since the 1960s, the pace at which it is rising has slowed since the early 2000s, and there has been no significant increase in the commonness of obesity from 2003 to 2011 (3).

Even though the rate of obesity's frequency has slowed, its prevalence has caused a rise in several other diseases. Heart disease, which has multiple causes, is strongly linked to obesity and is the number one cause of death in America (4). According to the Center for Disease Control and Prevention, the amount of deaths caused by heart disease increased by 3% from 2011 to 2014 (5). Type two diabetes is also strongly linked to obesity and accounts for 90-95% of all occurrences of diabetes. From 1995 to 2007, the prevalence of diabetes increased by 90% with roughly 9 in 1000 people being diagnosed with the disease (6). In 2012, 1.5 million deaths were directly due to diabetes and in 2014, 422 million people worldwide were diagnosed with it (7). Obesity has also caused an increase in the amount of total joint replacement surgeries that have been done. A retrospective study conducted in 2007 looked at the correlation between obesity and total knee replacements in adults aged 18 to 59 years old. It was found that from 2002 to 2004, 72% percent of the patients were obese with another 21% of them being overweight (8).

Obesity can cause many medical complications and has many causes with improper nutrition being one of the leading causes and one of the most common. An average American diet is calorie-packed with added preservatives, sugar, fat, and salt. In 2009, it was found that 67.5% of Americans ate less than two servings of fruit a day with 73.7% eating less than three servings of vegetables daily (9) when it is recommended that a person has four servings of fruit and five servings of vegetables a day (10). Most of the issue with nutrition relates to economics. Many fast food establishments make unhealthy food inexpensive and quickly obtainable, which is optimal in the fast-paced lifestyle most Americans live. Other restaurants add to this problem by over-portioning or by offering all-you-can-eat buffets where people try to get the most for their money (11).

Socioeconomic status (SES) can also play a role in becoming obese, even though many of the plethora of studies to date have conflicting results. One study found that families with an income closer to $75,000 were more likely to eat fast food than families with an income of $20,000. This could be due to fast food being seen as a luxury item or because they work more hours and have less time to cook (12). Another study found that men who make more money are more likely to be obese whereas women who make more money are less likely to be obese (13). Obesity also becomes a financial burden to the individual and the healthcare system. The medical complications an obese person incurs costs the healthcare system about 25% more than a person of normal BMI (14).

Another common cause of obesity is sedentary lifestyle. Americans are not as active as they used to be for a variety of reasons. Technology is a major contributor because people can do more with less effort. Technology has increased productivity, made once laborious jobs easier, and has made the world more connected, but it has also caused people to not be as active (15). However, there are many other developed countries in the world who do not struggle with obesity as much as the United States even though they have the same technology. In 2004, it was found that 33.1 percent of Americans aged fifty and older were obese as compared to an average of 17.1 percent determined from ten European countries (16). One possible reason for this is that many American cities and communities were constructed during an “automobile era” so they are not designed to encourage walking. In addition to this, fast food was originated in the United States and has become a large part of the American lifestyle. Contrarily, European countries generally do not promote eating fast food as frequently (17).

While the majority of people become obese due a sedentary lifestyle and improper nutrition, genetics also plays a role in obesity, and obesity can be a symptom of a genetic disorder. There are several biochemical pathways that lead to energy expenditure within the body, and one prominent one is the leptin-melanocortin pathway (Figure 1). When there is a single gene defect in this pathway, it causes severe obesity in humans. It was found that a homozygous frameshift mutation in the *lep* gene prevents a truncated protein from being secreted. This deficiency is also associated with hypothalamic hypothyroidism, which also leads to obesity (18). There have been nine loci that are identified and associated with hereditable forms of obesity with another fifty-eight loci contributing to polygenic obesity. One of these loci is found on chromosome 9, which caused a sensitivity to a moderately high fat diet resulting in obesity. Another loci on chromosome 15 has the same effect. A loci

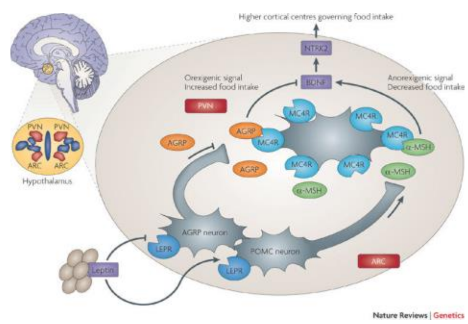

Figure 1: An example of the leptin-melanocortin pathway (69).

found on chromosome 4 is found to be responsible for the phenotype associated with dietary obesity caused by the other two loci (19). It has also been found that obesity can be heritable anywhere from 40% to 70%, which is similar to the heritability of height (20).

Fat Metabolism

There are three different types of adipose (fat) tissue, with the most common being white adipose tissue (Figure 2). White adipose tissue is mainly used for storage and is made up of unilocular adipocytes that have a large lipid droplet. This type of fat is what enables organisms to survive for longer periods of time without eating constantly (21). It also has less vascularization and fewer mitochondria and is also associated with adverse medical conditions like type II diabetes and insulin sensitivity (22). A second type of adipose tissue is brown adipose which is considered to be a specialized organ involved in thermogenesis, or the homeostatic mechanism for body heat (21). These cells are highly vascularized and have many mitochondria that contain uncoupling protein-1. Uncoupling protein-1 helps make heat by short circuiting the electrochemical gradient that drives ATP synthesis, resulting in energy usage and low energy production. The last type of adipose is beige adipose, which is white adipose that gets converted into brown adipose due to stimuli like extremely cold temperatures (23).

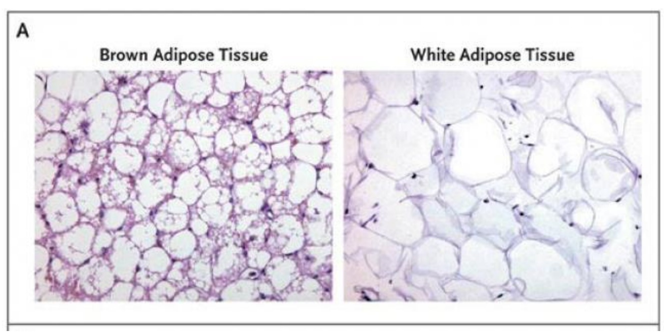

Figure 2: An example of brown adipose tissue (left) and white adipose tissue (right) (70).

The combination of a sedentary lifestyle and a calorie dense diet is the main cause behind obesity. By consuming more fuel and expending less energy, the body converts the unused energy into adipose tissue through a process called lipogenesis. This process converts carbohydrates into triglycerides and takes place mainly in the liver (24). Insulin helps signal the uptake and storage of glucose in the blood into muscle, liver, and adipose cells. It does this by activating one of two different pathways, either by pyruvate dehydrogenase (PDH) dephosphorylation or by acetyl-CoA carboxylase (ACC) conversion. PDH dephosporylation helps produce acetyl-CoA which can then be converted by ACC conversion to make malonyl-CoA that allows for more carbon atoms to be available to make larger fatty acids. Glucagon acts as an antagonist to insulin by increasing phosphorylation and inhibiting the previous two pathways. This results in the triglycerides being released from stored fat to be used as energy for the body (25).

Fat molecules can also be created using the de novo pathway (Figure 3). In this mechanism, acetyl-CoA is created from lactate that was derived from the glycolysis product, pyruvate. Acetyl-CoA is converted to malonyl-CoA by carboxylation using ACC. Malonyl-CoA is then acted on by fatty-acid synthase (FASN) to create long fatty acid chains (26). While the body is able to make new fatty acids for metabolic function, it is not the preferred method of the body. Most of the fatty acids used by the body come from the diet because it is easier to obtain them rather than making them from scratch (28). However, even with this preference, fatty acids can

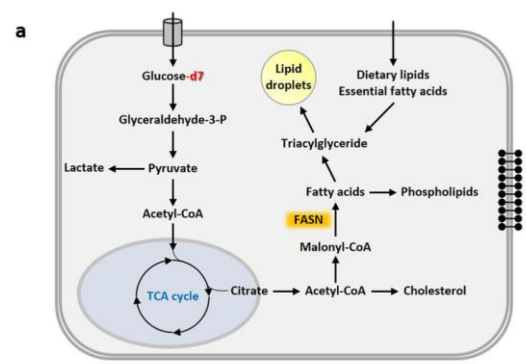

Figure 3: Mechanism of de novo lipogenesis (71).

build up quickly in the body because it will more readily use glucose. Fatty acids obtained from the diet contribute to about 35-50% of the total energy supply of the body (28). Phospholipid biosynthesis is an important part of fat metabolism as well because they are involved in the absorption, transportation, and storage of lipids. Phospholipids can be made through methionine synthesis (29).

While lipogenesis leads to the production of lipids, lipolysis is the opposite process. Lipolysis is the pathway in which triacylglycerol that is stored in cellular lipid droplets is catabolized. This is done by hydrolytically cleaving the triacylglycerol molecules into non-esterified fatty acids that are used for energy, lipid and membrane synthesis, or cell signaling (30). Triacylglycerol is found mainly in white adipose tissue and it serves as a major energy reserve. When energy is needed by the body, white adipose tissue increases the rate of triacylglycerol hydrolysis in order to produce diacylglycerol and subsequently monoacylglycerol, releasing a fatty acid in each step. Monoacylglycerol is then hydrolyzed one last time to release the last fatty acid and glycerol (31).

Another important part of fat metabolism involves appetite suppression which can be caused by several factors, like gastric expansion and serotonin levels. Adipose tissue contributes to appetite suppression by producing a hormone called leptin. This hormone is able to communicate how full a person's stomach is to the central nervous system (32). It does this by activating the release of serotonin. Serotonin is a hormone in the body that is produced from tryptophan after eating carbohydrate dense foods. It is associated with feelings of happiness and affects appetite suppression by activating neurons and melanocortin-4 receptors to suppress hunger and by blocking other neurons that would increase appetite (33).

Combating Obesity with Supplements

Due to the health complications that obesity causes, there has been extensive research into its prevention. One common suggested strategy is to increase the amount of exercise people receive. It is recommended that adults get at least thirty minutes of moderate-intensity exercise every day (34). Another means to combat obesity is to change one's diet to incorporate more fruits and vegetables as well as staying away from calorie-dense foods (34). However, with the time constrictions and the lifestyle previously mentioned, a lot of people do not exercise or do not change their diet. In response to this, they turn to chemical supplements to help lose weight. Supplements like Hydroxycut® and Contrave® are popular over-the-counter and prescription weight loss pills, respectively. Contrave® has to be prescribed by a doctor and its side effects include, but are not limited to, depression, seizures, and glaucoma (35). Hydroxycut® has similar side effects and has been shown to cause hepatotoxicity that, in one case, led to death (36). In 2009, the FDA warned against the use of Hydroxycut® due to the risk of liver damage (37).

Due to the adverse effects that chemical supplements have, many consumers look for a more natural option. There are several supplements that have become popular through social media and were tested in this study. The reason for testing them stems from a lack of evidence to support the claims from various celebrities and talk show hosts. Four compounds were tested in this study, with the first being raspberry ketone. Raspberry ketone is an ethanol-soluble, volatile phenolic compounds found naturally in raspberries, and is responsible for the “raspberry” aroma. In 2012, raspberry ketone seemed to be the newest trend in weight loss supplements as they demonstrated potential anti-obesity properties (38). It is thought that the ketones affect a protein in the body called adiponectin by upregulating its production (39). Adiponectin affects fat metabolism by enhancing insulin sensitivity in muscle tissue, to allow for a greater intake of glucose. It has also been found that people who are obese have less of the adiponectin hormone than people who are not obese, and that losing weight causes an increase in the hormone. This is why many think it might contribute to increasing metabolism (40).

While raspberry ketones have been heavily promoted for weight loss, there are no human studies that demonstrate their effectiveness or confirm their effect on adiponectin. Several studies in mice and *in vitro* have shown the potential of raspberry ketones to cause weight loss. One study fed mice a high fat diet for five weeks and then added either 500, 1000, or 2000 mg/kg of raspberry ketones for an additional five weeks. They found that the mice fed 1000 and 2000 mg/kg did not gain weight (41). These studies have also shown that the amounts of the compound used seemed to have no ill side effects in the mice (38). However, one study determined that the lethal dose of raspberry ketones was 1300 mg/kg for male rats and 1400 mg/kg for female rats (42). While there is no official dose for the compound due to no human studies, most supplement companies suggest a person take 100-200 mg/day. This provides another issue with commercially available raspberry ketones in that the majority of it is synthetically manufactured. The amount of raspberry ketones that are in a raspberry is so minute that it would take roughly ninety pounds of raspberries to make one recommended dose (39).

Another compound that is very popular due to media exposure is *Garcinia cambogia* (*G. cambogia*). *G. cambogia* is a plant from South Asia that has risen to weight loss fame as a result of its extract, hydroxycitric acid (HCA) (43). HCA is a derivative of citric acid and is thought to help with weight loss by acting as a competitive inhibitor to the enzyme adenosine triphosphate-citrate-lyase (44). This enzyme is important in the de novo synthesis of fatty acids and cholesterol and, by inhibiting its functions, HCA blocks one pathway used to create fat in the body (45). It is also thought the HCA can help someone lose weight by increasing the release of or the availability of serotonin in the brain which would cause appetite suppression (46). Unlike raspberry ketones, there have been human trials to test the weight loss function of HCA. It was found that it may help with weight loss but the effects were reported to be very small as compared to a prescription weight loss medication. Most of the people in the trials also reported adverse effects like nausea, headache, and gastrointestinal symptoms. There still has been no optimal dosage found for the compound, yet most of the supplement companies recommend a dose of 2400mg per day (47).

Another popular plant derivative used for weight loss is cayenne from red peppers. The active ingredient in cayenne is capsaicin (48). Capsaicin works by binding to a sensory protein called the transient receptor potential vanilloid subfamily member 1 (TRPV1), which is responsible for the burning sensation of peppers. However, the receptor is also connected to nerves that when stimulated, activates the production of uncoupling proteins. This leads to the breakdown of fat through increased tissue heat production (49). Several studies regarding the use of cayenne as a weight loss supplement have been done; however, many of these studies have conflicting results. A study from the American Journal of Clinical Nutrition found that humans given an oral dose of 6mg of cayenne per day over a twelve week period saw a decrease in abdominal fat with no other changes to lifestyle. However, there was no reduction in body weight or in body fat percentage (50). Another study looked at whether cayenne could be used to maintain body weight after weight loss. They found that there was no difference in how much weight was regained between the placebo and trial groups (51). Due to the variability in previously done studies, there is a still a need to look into using this compound for weight loss. There are also some adverse side effects reported from taking the supplement such as stomach irritation, ulcers, and heart burn. While supplements are regulated to make sure they are not lethal, they are not regulated in the same way that drugs are. The FDA is responsible, in part, for regulating drugs and making sure enough clinical testing has proved its safety, dosage, and effectiveness. Due to the lack of FDA regulation for supplements, most supplements do not have an actual defined dose nor are their effects supported by evidence. Therefore, for cayenne, most companies have a recommended dose of 3450mg per day (48).

Some vitamins are also becoming popular in the battle against weight loss, including vitamin B12. This is a water soluble vitamin that is found in most of the foods people eat. It has several functions in the body such as red blood cell formation, DNA synthesis, and is involved in neurological function. A malabsorption or deficiency in B12 can lead to anemia and neurological disorders (52). B12 has been found to increase metabolism by helping to balance the amount of methyl radicals needed for phospholipid biosynthesis. It does this through methionine synthesis where it acts as a cofactor for methionine synthase and L-methylmalonyl-CoA mutase (53). Further in this pathway, succinyl-CoA is formed through the degradation of propionate which is essential in fat and protein metabolism (52). It was found that a deficiency in B12 also caused an increase in C15 and C17 fatty acids; therefore, it is thought that excess B12 could be used to further increase fat metabolism to reduce the amount of fat in the body (54). As vitamin B12 has been extensively studied, a large amount of data exists on effective doses; therefore, the recommended daily dosage is 2.4 micrograms a day for a human (52).

Fruit flies as a model organism

The fruit fly, *Drosophila melanogaster (D. melanogaster)*, has been a popular, cost-effective model organism for studying human diseases. Many genes and biochemical pathways are conserved between humans and fruit flies with around 75% of human disease genes having orthologs in the fly genome (55). With regards to fat metabolism, *D. melanogaster* has similar cell types and organ anatomy to mammals that are used in lipid metabolism and homeostasis. Fruit flies store lipids as triacylglycerol (TAG) and their adipocytes contain lipid droplets like mammalian cells. *D. melanogaster* has a structure called a fat body that, in conjunction with other secreting cells, act similarly to a liver in humans. While humans produce insulin through the β-cells of the pancreas to regulate carbohydrate and lipid metabolism, fruit flies do not have a pancreas at all. Instead they have corpora cardiac cells which are located in the larval ring gland. These cells produce a glucagon-like adipokinetic hormone (AKH) and behave similarly to the α-cells of the pancreas. Flies that lack the AKH receptor cannot respond to AKH and have increased fat stores whereas an overexpression of AKH would decrease fat stores (56). In addition to this, *D. melanogaster* also produce seven insulin-like proteins through insulin-producing cells (IPCs), which are analogous to β-cells and are localized in the central brain (57). Micro RNA Mir-14 is also a gene of interest because it has been found to regulate fat metabolism. Flies that lacked Mir-14 were found to have increased triglyceride levels as opposed to those with normal or overexpressed values (58).

Due to these similarities, *D. melanogaster* is an effective and appropriate model organism for studying lipid metabolism, and for testing the effects of natural dietary supplements of fat metabolism (57). *D. melanogaster* uses the same set of lipogenic enzymes involved in TAG *de novo* biosynthesis from fatty acids that mammals do. The two also share the same transcription factors that vary slightly in function (59). For example, in mammals, the transcription factor SERBP is important in regulating cholesterol and fatty acid synthesis and its activity is downregulated in the presence of cholesterol (60). In the fruit fly, this same transcription factor is mainly responsible for regulating fatty acid synthesis and its negative inhibitor is phosphatidylethanolamine (61).

Goals and Objectives

In this study, we had two main goals. The first was to look at the effects of four "natural" dietary supplements on fat metabolism: raspberry ketones, vitamin B12, *Garcinia cambogia*, and cayenne. These compounds have been chosen either for popularity, as in the case of *Garcinia cambogia* and raspberry ketones, or for claiming to reduce fat by increasing metabolism, as in the case of cayenne and B12 (62). The reason for this part of the project is to observe the effects of these four compounds because their effectiveness is unknown. This part of the project will help determine whether these compounds do in fact increase fat metabolism.

The second goal was to test the supplements on two genetic lines that had mutations resulting in increased fat storage. The first line had a mutation that prevented the *Drosophila* adipokinetic hormone (dAKH) receptor from performing its function of responding to the hormone AKH which is analogous to glucagon (56). The second line had a mutation in the gene that produces Mir-14, a regulator of insulin levels, which also has increased triglyceride levels (58). This part of the project was done to observe the effectiveness of the compounds in flies that have a disruption in their fat metabolism pathway and to observe any possible links between the compound mechanisms and fat metabolism.

Material and Methods

Fly Nutrition

Sixteen types of fly food were made, each containing one of the supplements, with four different doses tested for each supplement. Each supplement was dissolved in water and then mixed with standard cornmeal agar with the exception of raspberry ketone, which is not water soluble. It was dissolved in ethanol and, as a result, raspberry ketones was tested at three concentration and ethanol to control for any effect of the solvent. Concentrations (Table 1) were based on recommended human doses (39, 47, 48, 52), plus at least one higher and one lower, and all of the food was pigmented using food coloring. Three different lines of flies were used: wildtype, Mir-14 mutants (lack the mir-14 gene), and dAKH mutants (lack the AKH receptor gene). The fly lines were maintained on standard cornmeal agar media at room temperature when not being tested. Wild-type flies were separated by gender and kept on supplemented and unsupplemented food for one week. All concentrations were tested for all supplements for both genders of wildtype flies. *G. cambogia* and cayenne were tested at every concentration for the Mir-14 and dAKH fly lines but only males were used in these trials. Only the highest concentrations were tested on males in the Mir-

Table 1: Concentrations of supplemented fly food. The highlighted cells are the "recommended" dosage for the respective compound.

Cayenne Concentrations (g/mL)	B12 Concentrations (µg/mL)	Raspberry Ketone Concentrations (g/mL)	Garcinia Cambogia Concentrations (g/mL)
0.457	6.000	2.000	1.500
4.57E-04	3.00E-05	0.200	0.150
4.57E-07	3.00E-07	0.020	0.015
4.57E-08	3.00E-09	Ethanol Control: 0.2%	1.50E-03

14 and dAKH fly lines for raspberry ketone and vitamin B12. No females were tested with dAKH or Mir-14 mutants for any compound.

Triglyceride measuring

Five flies were homogenized from each concentration for each trial. Each group of flies was homogenized in Phosphate Buffered Saline with 0.05% tween (PBS-tween) using a sterile plastic disposable pestle. Fat metabolism was assayed using a triglyceride kit (Sigma) that used a colorimetric assay to determine triglyceride levels. The assay was read at 540nm, with higher absorption readings correlating to a greater number of triglycerides. All fly lines and compounds were tested in triplicate to obtain an average absorbance. A standard curve was prepared and used to convert the absorbance into mg/mL of triglyceride. A BSA assay was used to determine protein concentration. This provided a standard to ensure that a similar amount of material had been extracted in each trial.

Statistical Analysis

Linear regression was performed to determine if there was a correlation between compound concentration and triglyceride level. A one-way ANOVA was used to compare mean triglyceride levels and different supplement concentrations, and a two-sample t-test was used to compare triglyceride levels between individual concentrations and the respective control group.

Results

To confirm the flies were eating the food, food coloring was added to all diets, including the control. No difference in food consumption was observed for any diet (Figure 4). For raspberry ketone, an ethanol control was done to account for raspberry ketone only being soluble in ethanol. This control was only done with raspberry ketone because it was the only compound that was not soluble in water. The wildtype males saw an initial decrease in triglyceride levels with a concentration of 0.02g/mL raspberry ketone followed by an increase as the concentration of raspberry ketone increased. The decrease observed was not significant (Figure 5). The female wildtype flies showed a stark decrease from the control group with the concentration of 0.2g/mL raspberry ketone. This was followed by an increase of triglyceride levels with the 2g/mL dose of raspberry

Figure 4: Wildtype female kept on colored food.

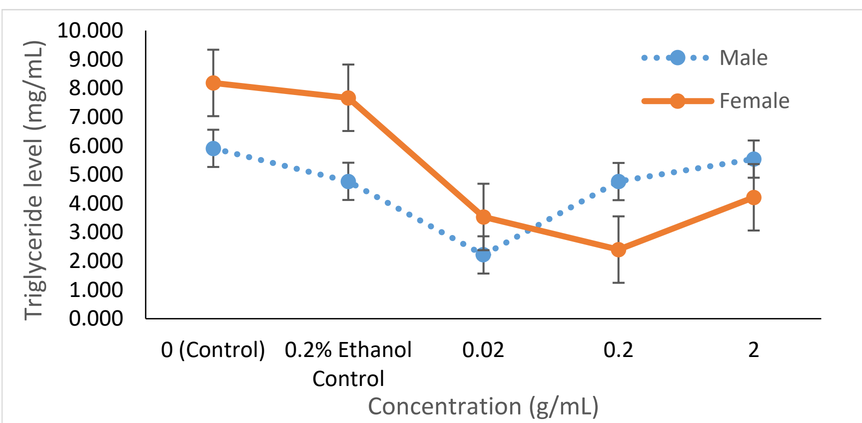

Figure 5: Effect of raspberry ketone on triglyceride levels in male and female wild-type *Drosophila melanogaster*. Triglyceride levels are given in mg/mL. The results of the linear regression for males and females were not significant (r^2=0.0068 and r^2=0.7986, respectively). The results of the ANOVA for both the female and male data was not significant (p=0.066 and p=0.324). The results of the t-test comparing each concentration to the control individually were not significant (p>0.05).

ketone (Figure 5). The decrease between the 0.2g/mL concentration and the control or ethanol control group was not significant ($p = 0.0586$ and $p=0.667$, respectively).

The wildtype males treated with vitamin B12 showed no correlation between triglyceride levels and supplement concentration. The female wildtype flies also lacked a correlation between triglyceride levels and supplement concentration (Figure 6). However, these trials best display a trend noticed amongst all of the wildtype trials in which male flies tend to have less triglyceride levels than the females. While some exceptions can be seen (Figure 5 and 8, higher concentrations), for the majority of the trials, the males had less triglycerides. This difference was not statistically significant ($t = 1.010$, $df = 22$, $p = 0.323$). The exception was male and female triglyceride levels were statistically different in the vitamin B12 trials ($t = 6.410$, $df = 28$, $p < 0.001$).

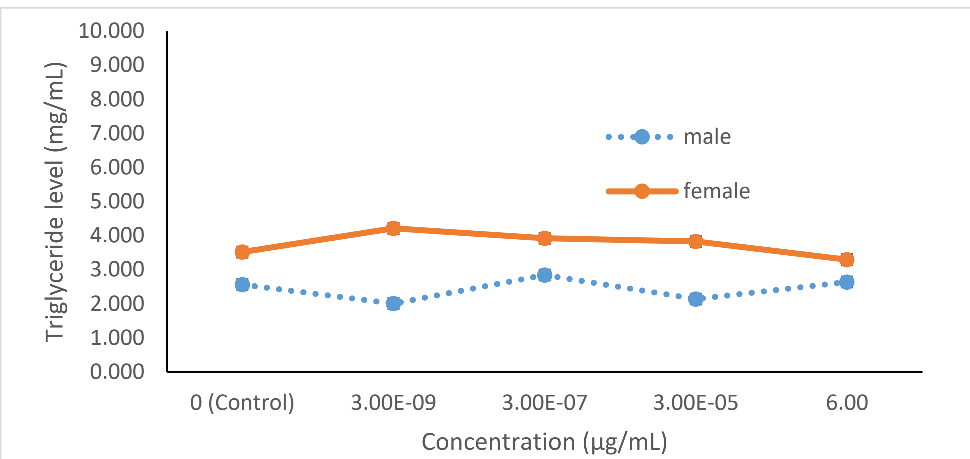

Figure 6: Effect of B12 on triglyceride levels in male and female wild-type *Drosophila melanogaster*. Triglyceride levels are given in mg/mL. The results of the linear regression for males and female were not significant ($r^2=0.0171$, $r^2=0.1317$, respectively). The results of the ANOVA and the t-test were not significant ($p> 0.05$ for both).

Male wildtype flies treated with *G. cambogia* showed an increasing trend in triglyceride from the control group as the concentration of *G. cambogia* increased except at the highest concentration (1.5g/mL), where there was a decrease in triglyceride levels but neither one was significant (Figure 7). The female wildtype flies also exhibited the same trend as the males (Figure 7). Statistical analysis showed there was no correlation between triglyceride levels and compound concentration (linear regression, r^2=0.0499) and no significant differences between the mean triglyceride levels of the flies treated with the compound and the control group (F_s = 2.227, p = 0.139).

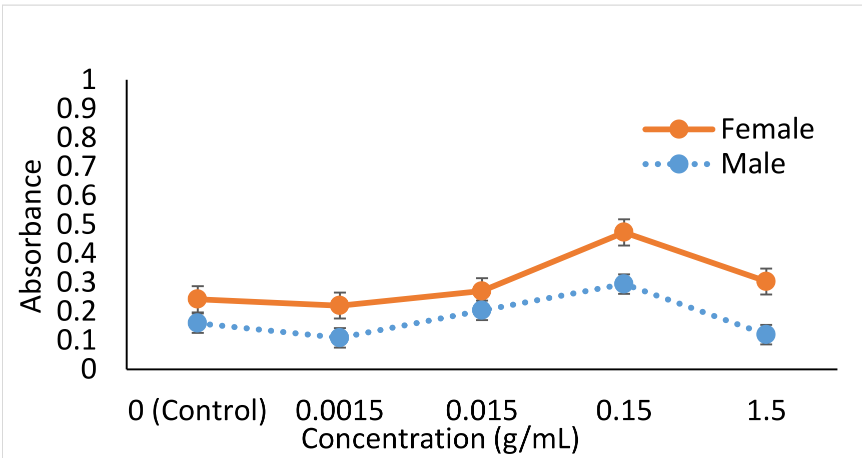

Figure 7: Effect of *Garcinia cambogia* on triglyceride levels in male and female wild-type *Drosophila melanogaster*. Triglyceride levels are presented as absorbance readings. The results of linear regression for the males and females were not significant (r^2=0.0499 and r^2=0.3477, respectively). The results of the ANOVA and the t-test were not significant (p>0.05 for both).

The wildtype males which were treated with cayenne showed a very strong trend in which, as the concentration of cayenne increased, triglyceride levels decreased (Figure 8), with a significant difference in triglyceride levels being observed at highest concentration of cayenne (t-test, p=0.014, df=4). This concentration of cayenne resulted in a change of -65.1% of triglycerides (Figure 10). The 0.457g/mL concentration was the only concentration that provided a significant result with the wildtype males. The wildtype females showed no correlation between triglyceride levels and cayenne concentration (Figure 8).

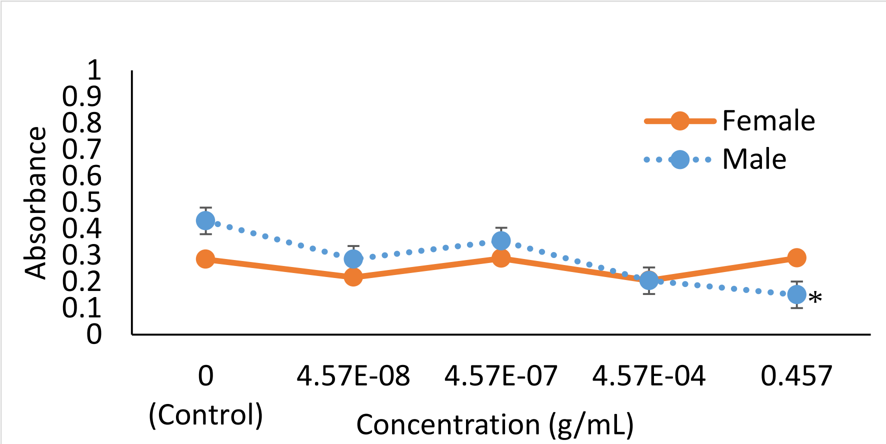

Figure 8: Effect of cayenne on triglyceride levels in male and female wild-type *Drosophila melanogaster*. Triglyceride levels are presented as absorbance reading. The results of the linear regression were not significant for males and females (r^2=0.8123 and r^2=0.0002, respectively). The results of the t-test showed that the difference between the control trials and the 0.457g/mL trials were significant (p = 0.014). All other t-test results were not significant (p > 0.05) and the ANOVA results were also not significant (p > 0.05). (*=p<0.05)

For the genetic mutants, only the males were tested. This is due to the female flies having differences in triglyceride levels, depending on what stage of the reproductive cycle they are in, which leads to a lot of variability. Also, only the highest concentrations were tested for raspberry ketone and vitamin B12 whereas all of the concentrations were tested for cayenne and *G. cambogia*. This is because *G. cambogia* and cayenne showed the strongest association of all the compounds after linear regression in the male wildtype flies (r^2=0.0499, r^2=0.8123, respectively).

The dAKH males treated with *G. cambogia* showed an increasing trend in triglyceride levels as the concentration increased (Figure 9) but it was not significant (F_s = 0.548, p = 0.705). The Mir-14 males showed this same trend (Figure 9), but it was also not statistically significant (F_s = 0.441, p = 0.776). With the dAKH males treated with cayenne, a significant increase in triglyceride with the lowest concentration was observed (t = 2.793, df = 4, p = 0.049). The three highest doses of cayenne also had increased triglyceride levels, compared to the control, but were not to the same extent as the lowest dose (Figure 10), and none were significant(t-test, p>0.05, df=4). The Mir-14 males

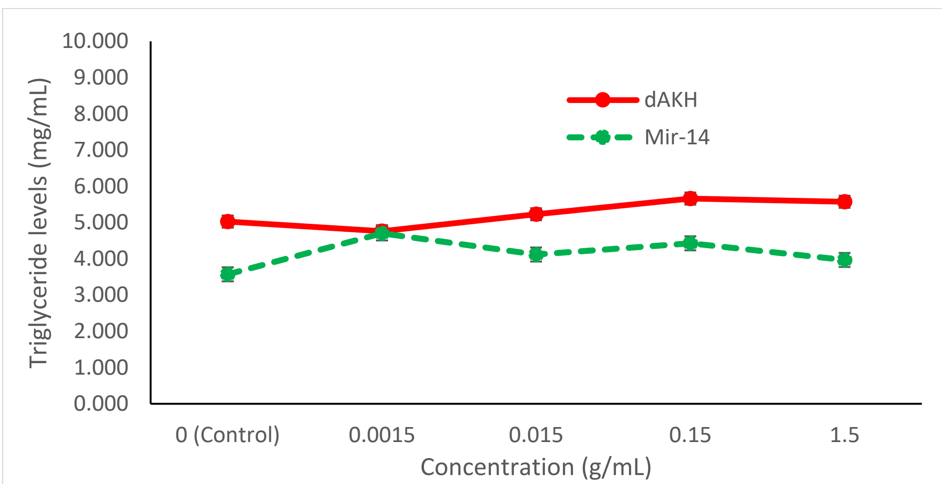

Figure 9: Effect of *Garcinia cambogia* on the dAKH and Mir-14 *Drosophila melanogaster* fly lines. The results of linear regression were not significant for either the dAKH or the Mir-14 mutants (r^2 = 0.7074 and r^2 = 0.035, respectively. The results of the ANOVA and the t-test were not significant for both fly lines (p > 0.05 for both).

treated with cayenne had a slight decreasing trend in triglyceride concentration as the cayenne concentration increased (Figure 10) but it was not significant.

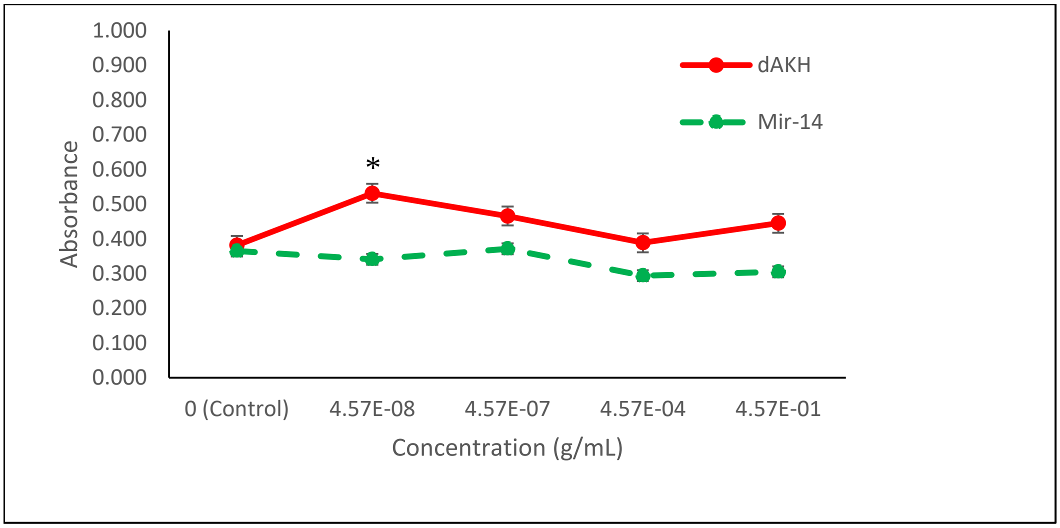

Figure 10: Effect of cayenne on the dAKH and Mir-14 *Drosophila melanogaster* fly lines. The results of the linear regression were not significant for either the dAKH or Mir-14 mutants (r^2 = 0.0016 and r^2 = 0.5721). The lowest dose of cayenne provided a significant increase ($p = 0.049$) for the dAKH mutants. All other results of the t-tests and the results of the ANOVA for dAKH and Mir-14 mutants were all not significant ($p > 0.05$ for both). (*=$p<0.05$)

The Mir-14 males treated with the highest dose of raspberry ketone showed a decrease in triglyceride levels, as did the dAKH males. The Mir-14 males showed more of a decrease than the dAKH males (-27.5% vs. -8.7%; Figure 10), however, neither one was significant. The Mir-14 males treated with the highest dose of vitamin B12 showed a slight increase (7.9%; Figure 11), whereas the dAKH males showed a slight decrease (-2.8%; Figure 11). However, neither one was significant.

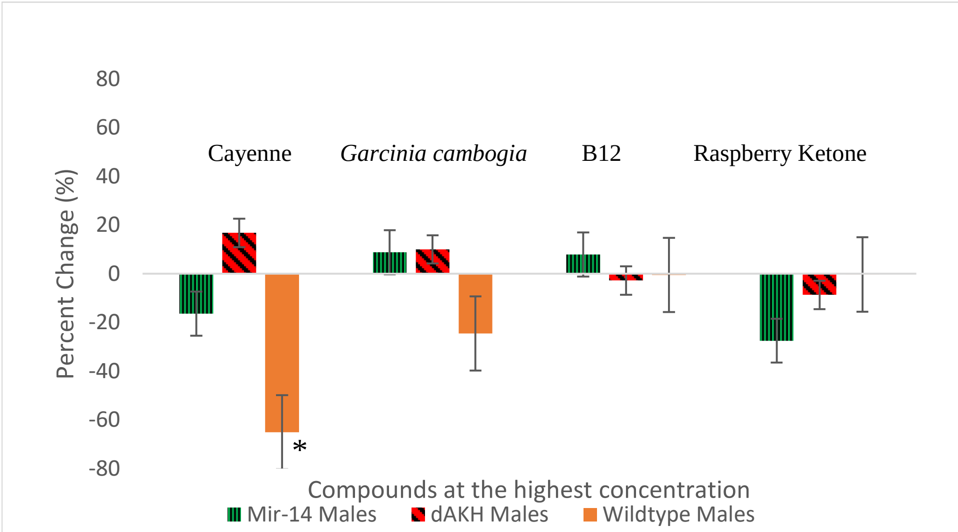

Figure 11: Percent change of the male flies treated with the highest doses of each compound for each fly line. Wild-type males treated with 0.457 g/mL shows a significant percent change of -65.1% (p=0.014). (*=p<0.05)

Discussion

Of all the compounds tested, cayenne was the most effective at reducing triglyceride levels. The male wildtype flies showed a strong decreasing trend as the concentration of cayenne increased. The highest concentration of cayenne (0.457g/mL) provided a significant decrease of triglyceride levels of 65.1% less than the control (p-value=0.014). This indicates that cayenne may increase fat metabolism and it agrees with some of the literature that supports cayenne's use as a weight loss supplement (48-51). One study showed that cayenne ingestion of 6mg/day resulted in some abdominal fat loss in humans as compared to the placebo group over a twelve week period but was not significant (50).

The female wildtype flies did not have a decreasing trend, but this could have been due to differences in what stage of the reproductive cycle the females were in. However, one concern is the concentration of the compound needed to produce a significant result. The normal dose of cayenne for a human as recommended by supplement companies is 3450mg per day (48). For this study, the dose was scaled to account for the size difference between a fruit fly, which is around 10mg, and an average human, which is about 81kg. This makes a fly about eight million times smaller than a human. The actual dose the fly was supposed to receive was 4.57E-07 g/mL as compared to 0.457g/mL which produced a significant result. This concentration is one million times stronger than what the fly is supposed to receive. While it was not lethal to a fly, it could cause severe gastrointestinal issues in humans if they were to ingest that much cayenne. This is because cayenne's active ingredient, capsaicin, activates the sensory protein TRPV1 which is responsible for the burning sensation of peppers (66). This would enhance the effects of cayenne by causing extreme cases of heartburn, kidney and liver damage, and gastrointestinal peristalsis (49).

Overall, most of the compounds tested did not significantly lower triglyceride levels. With the wildtype female trials, there is a large drop in triglyceride levels between the control group and the 0.2g/mL concentration, which was close to being significant ($p=0.0586$) but this difference is reduced when compared to the ethanol control group ($p=0.667$). This suggests that the ethanol may have had a contributing effect in helping decrease triglyceride levels. Vitamin B12 showed almost no change in both the wildtype males and females. There was an observed trend in which males had less triglycerides overall than the females did. This trend was observed among most of the trials; however, upon statistical analysis, it was found that this difference was not always significant. This finding does agree with what has been found in previous studies of fruit flies (63). One study found that female fruit flies ate more often than males to be able to reproduce and have adequate nutrition (64). Female fruit flies are also able to mate with several different males and store the sperm cells until they are to lay eggs, which is shortly after copulation. The females can repeat this process several times over the course of its life (65). Due to this variability of lifecycle involving reproduction, which will affect fat (triglyceride) levels, only male flies were tested in the mutants.

G. cambogia showed a slightly decreasing trend in triglyceride levels in the male wildtype flies at the highest concentration (1.5g/mL) although this was not significant ($p=0.482$). This supports what other researchers have found in that *G. cambogia* may decrease triglyceride levels but not in a significant way (47). The female wildtype flies showed relatively no change with any concentration of *G. cambogia* tested. Due to the lack of significant changes in triglyceride levels, it does not appear that raspberry ketone, vitamin B12, or *G. cambogia* are effective as weight loss supplements.

For the second part of this experiment, males from the dAKH and Mir-14 fly lines were tested against each compound. Both of these lines have mutations that increase triglyceride levels so that if the compounds did have an effect, these flies would be most likely to show it. It also provides an analogy to how these compounds might affect a genetically overweight human. The Mir-14 males when given the highest dose of cayenne showed a percent decrease in triglycerides of -16.4%, which was not significant. However, the dAKH males showed a significant increase (p-value=0.049) with the lowest dose of cayenne (4.57E-08 g/mL). The highest concentration of cayenne, while not significant, also showed a percent change of +16.8%. This could indicate that cayenne affects fat metabolism through the dAKH pathway. This is hypothesized because both the wildtype males and the Mir-14 males exhibit decreasing trends but the dAKH males showed a significant increase. The increase we saw in the dAKH mutants was unexpected because of the decreasing trends we saw in the wildtype and mir-14 fly lines.

The dAKH mutants have a mutation where they lack the receptor for recognizing AKH. This causes them to have increased triglyceride levels as they are not able to metabolize their fat stores to be broken down into glucose in response to AKH (56). Cayenne is also responsible for stimulating the production of uncoupling proteins which leads to increased heat production (49). This protein is uncoupling protein 1 (UCP1) in humans which is regulated through norepinephrine released from sympathetic terminals and acts through beta-adrenoceptors and cAMP (67). Fruit flies have a similar protein called DmUCP5, which acts like UCP1 (68). It is possible that the cayenne stimulates this

DmUCP5 pathway but, because the mutants are not able to mobilize their fat stores for energy, they eat more than normal. The excess food they ingest then becomes transformed into fat that cannot be used and the cycle continues. All of the dAKH males treated showed an increase of triglyceride as compared to the control with the lowest dose of cayenne providing a significant result. Further inquiry would be needed into this hypothesis and the possible link between the dAKH pathway and cayenne's effect.

Both the Mir-14 and the dAKH males were tested at the highest concentration for raspberry ketone (2g/mL) and showed to have a very minor percent change of -27.5% and -8.7%, respectively ($p = 0.071$ and $p = 0.474$, respectively). Vitamin B12 also had a very minor effect on the Mir-14 and dAKH flies at the highest concentration (6μg/mL). With *G. cambogia*, triglyceride levels were marginally increased in both the Mir-14 and dAKH flies as the concentration increased. The fact that similar trends were observed for both could be indicative of a link between the Mir-14 and dAKH pathways where without one or the other, HCA from *G. cambogia* cannot be used to stop fat production through the de novo pathway. However, because the increases were not significant, there is not enough data to support that hypothesis.

Overall, the majority of the compounds tested did not significantly lower triglyceride levels. There was a trend seen between male and female wildtype flies in which male had less triglycerides as compared to the females. Cayenne also provided the strongest trend in the wildtype males that showed a decrease of triglycerides as the concentration of cayenne increased, with the highest dose of cayenne producing a significant decrease in triglyceride levels, similar to what has been observed in humans (50). Both mutant fly lines shared an increase in triglyceride levels, but were largely unaffected by any of the compounds. The dAKH males showed a significant increase with the lowest concentration of cayenne, which could be due to a link between the dAKH pathway and capsaicin’s effect on fat metabolism.

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