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The Effects of a High Saturated Fat Diet and a High Protein Diet on the Effects of Left-Ventricular Hypertrophy

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Abstract

Studies indicate that high protein consumption increases risk of cardiovascular disease, while high saturated fat consumption reduces risk. The effects of these diets on left-ventricular hypertrophy remain unknown. This study examined the effects of high protein and high saturated fat diets on left-ventricular hypertrophy in *C. elegans.* Two experimental groups mutated with left-ventricular hypertrophy (JM311 strain) were fed either high protein or high saturated fat diets, while two control groups of JM311 worms and N2 wildtype worms were fed OP50 diets. It was hypothesized that worms fed high fat diets would have less severe left-ventricular hypertrophy than the worms fed high protein diets. Egg viability assay results showed lower egg counts in JM311 control strains (16.33±0.31 eggs) compared to control N2 worms (34.67±0.92 eggs). The high saturated fat diet groups had higher egg counts (32.33±0.28 eggs) than high protein diet groups (26±0.36 eggs). The number of eggs laid by the high saturated fat worms was significantly higher than those of the high protein worms (p=0.03826). The number of eggs laid by both high saturated fats (p=0.65) and the high protein worms (p=0.1642) did not significantly differ from the N2 wildtype group suggesting that high saturated fat and high protein diets can enhance recovery rates in left-ventricular hypertrophy patients, with high saturated fat diets having a more significant effect on recovery rates. These results provide insights on potentially beneficial dietary approaches to clinicians treating patients with left-ventricular hypertrophy.

Keywords: C. elegans, Cardiomyopathy, Left-Ventricular Hypertrophy, Nutrition, Protein, Fats, Diet

1. Introduction

Left-ventricular hypertrophy (LVH), otherwise known as hypertrophic cardiomyopathy, occurs when the walls of the left ventricle, the heart's main pumping chamber, thickens, adding stress to the cardiovascular system. Blood pressure in the heart causes the walls to thicken to the point where the heart is not able to pump with enough force needed to circulate the blood properly. Nearly 1 in 5 people in the United States are affected by LVH (Cleveland Clinic, 2023). If left untreated, this can eventually lead to arrhythmias, heart failures, and other serious complications.

Caenorhabditis elegans are a species of worms used as model organisms for studying many human diseases due to their physiological overlap with humans including the digestive, nervous, and reproductive systems (Yen & Curran, 2016). It has been shown that of the 42 mutated human proteins that are associated with various cardiomyopathies, 35 proteins have orthologs or homologs in *C. elegans*, suggesting that *C. elegans* are a good model organism for left-ventricular hypertrophy (Benueb & Epstein, 2011). In addition, it has also been suggested that because *C. elegans* have a short reproductive cycle, complex multicellular structure, and shared genes with humans, they can be used to replicate dietary and metabolic changes, making *C. elegans* good organisms to evaluate the effects of diet on heart function (Benueb & Epstein, 2011; Yen & Curran, 2016).

Past research has shown evidence to support that high protein diets increase the risks of various arterial diseases (Zhang et al, 2020). After feeding Apolipoprotein E (ApoE) null mice with high-protein diets and measuring the amount of plaque in their body over two months, it was found that there was a significant increase in atherosclerotic plaque at the aortic root. This plaque build-up can cause the arteries to narrow, which leads to multiple cardiovascular diseases (CVD) like atherosclerosis (Zhang et al, 2020). LVH is a cardiovascular disease that is not caused by plaque buildup, suggesting that a high protein diet may not have the same effect on left-ventricular hypertrophy as other CVDs. Another common misconception is that consuming large amounts of fatty acids increases the risk of cardiovascular disease. However, recent data found that the number of unsaturated fats consumed by Swedish men was inversely proportional to that of the cardiovascular disease risk of each participant suggesting that high-fat diets may increase cardiovascular health, in contrast to prior belief (Trieu et al, 2021).

The severity of LVH in *C. elegans* has been measured by counting the eggs laid by gravid adults. In one study, the different properties of two new strains of *C. elegans* (Strain JM311 and VC1317) with mutations that replicate left-ventricular cardiomyopathy were investigated by comparing physiological properties to the wildtype N2 strain (AlKhaleefa, 2020). The JM311 strain was chosen because it contains the *lem-2*(ca19) mutation that results in premature cardiomyopathy, and had a measurable characteristic to track the severity of LVH. The effects of cardiomyopathy in *C. elegans* were compared to the N2 wild-type strain and it was found that the mutated strains' relative brood size was reduced when compared to that of the N2 wild-type strain. These results suggest that *C. elegans* with more severe LVH lay fewer eggs than those with less severe hypertrophy.

Diet has a large impact on the human body, especially for those with cardiovascular disease. Healthy eating not only allows for a longer life span, but also lowers risks of obesity, heart disease, type 2 diabetes, and cancer (Center for Disease Control, 2022). However, with LVH and other cardiovascular diseases, there is conflicting research about what patients should eat or avoid eating, making it hard for them to make healthy eating decisions. This study aimed to determine whether a high saturated fat diet or a high protein diet improved left-ventricular hypertrophy and its progression. These results will help patients navigate controversial information about the benefits or disadvantages of protein and saturated fat diets related to LVH. It was hypothesized that if *C. elegans* mutated with left-ventricular hypertrophy were fed a high protein diet or a high fat diet, the left-ventricular hypertrophy would improve in the group of *C. elegans* that was fed the high fat diet as opposed to the group that was fed the high protein diet because it has been shown that higher consumption of fats leads to an increase in cardiovascular health.

2. Materials and Methods

JM311 and N2 *C. elegans* were obtained from the Caenorhabditis Genetics Center (University of Minnesota). All other reagents were obtained from Millipore Sigma.

In order to evaluate the effect of the macronutrients on left-ventricular hypertrophy, this study uses two experimental groups, JM311 *C. elegans* that are fed either a high saturated fat (HF) diet or a high protein (HP) diet added to the standard diet of *E. coli* OP50. JM311 and N2 wildtype *C. elegans* were fed the standard diet of *E. coli* OP50 and used as a negative control to determine the number of eggs laid by healthy adult worms for each strain. The HF diet includes an addition of 37% palmitic acid and the HP diet includes the addition of 37% valine to the standard *E. coli* OP50 diet.

2.1 Plate Preparation and Stock Culture Maintenance

Nematode Growth Media (NGM) plates were prepared by adding 3 g of NaCl, 17 g agar, and 2.5 g peptone to a 2 L erlenmeyer flask. Distilled water was added until the total contents in the flask was 975 mL and the flask was sealed with aluminum foil and autoclaved for 20 minutes at 121°C at 15 psi. The contents of the flask were left to cool to 50°C and 25 mL of KPO_4 solution pH 6.5, 1 mL of $CaCl_2$, 1 mL $MgSO_4$, and 1 mL of 5 mg/mL cholesterol in ethanol were added to the flask and mixed. Using sterile technique, the NGM was poured into petri dishes until ⅔ full, and the plates were left for 2 days at room temperature to cool and check for contamination.

E. coli OP50 was cultured by inoculating 5 mL of Luria Broth (LB) with a frozen glycerol stock of bacteria. The

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newly inoculated tube was placed in a shaking incubator at 20ºC for 24 hours, then stored at 4ºC for later use. For the HF and HP cultures, *E.coli* OP50 was pipetted into a new tube, and valine (protein) or palmitic acid (fat) were added to a final concentration of 37%. The palmitic acid was dissolved in a very small amount of ethanol before addition.

To maintain stock cultures of JM311 and N2 *C. elegans* on the standard diet, *E. coli* OP50 was added to the center of an NGM plate and incubated at room temperature until a bacterial lawn formed. A chunk of agar from a plate of *C. elegans* was moved to a fresh plate using a sterile inoculation loop. Stocks were stored at room temperature and recultured every two weeks. They were disposed of by submerging the contents of the plate in 10% bleach for 10 minutes.

2.2 Egg Viability Assay

To synchronize the *C. elegans*, 50 µls of freshly prepared alkaline hypochlorite solution (1 ml bleach, 250 µl of 10M NaOH, and 3.75 ml sterile deionized H_2O), was pipetted onto an NGM. Gravid *C. elegans* from standard, well-fed culture stocks were chunked onto the plate, and left for 48 hours.

Another NGM plate was prepared with the corresponding high fat diet, high protein diet, or standard diet. A worm pick was gently scraped over the plate with OP50 in a small area and very quickly, the pick was moved to the prepped plate, and pressed onto the media until the worm moved away from the pick and onto the plate. This was repeated 10 times, for a total of 10 synchronized worms on each plate. The worms were left on the plate for 96 hours at room temperature. A dissecting scope was used to observe and count the number of eggs laid on the plate. Three trials of egg viability assays were conducted in order to accurately measure brood size and determine the severity of the condition of each worm group (AlKahleefa, 2020).

2.3 Statistics

Because only three trials were conducted, a non-parametric Mann-Whitney test was the choice of test to conduct. A Mann-Whitney test compares two groups and their medians in order to come up with statistical conclusions. In a Mann-Whitney test, the data values of both groups are combined and ranked from smallest to largest. From there, they are split again, with the ranks separated for each group. The sum of the ranks of each group are found and the U values for each group are found with this formula: U1 = n1n2+0.5n1(n1+1)-R1. The smallest U statistic is that and using a Mann-Whitney table, the U-statistics for the test is found using 5% significance value. This is compared to the minimum U-statistic calculated, and if the U-calculated is smaller than the U-statistic, then there is a statistical difference between the two data values.

However, due to technological advancements, Mann-Whittney tests can be conducted easily with Mann-Whitney calculators. In these calculators, the U-statistic and p-values are given. The p-value is the probability that the null hypothesis is supported by the data. The null hypothesis is always that there is no difference between the two data sets. Using a 95% confidence level, a p-values of 0.05 or lower indicates that the null hypothesis is not supported by the data, and that there is a significant difference between the two data sets.

One Mann-Whitney test was used in order to determine if the HF diet group laid significantly larger amounts of eggs than the HP diet, allowing us to come to a conclusion about which diet was the better diet in improving the worm's LVH conditions. Two more Mann-Whitney tests were conducted, in order to test whether there was a significant difference between the N2 strains and the HF and HP diet groups, in order to determine whether the two diets were able to improve the condition on the worms so significantly that there was no statistical difference between the median eggs laid by the healthy N2 strain and two diet groups.

3. Results

Control N2 *C. elegans* on the standard diet laid 34.67 ± 0.92 eggs per plate while JM311 *C. elegans* on the standard diet laid 16.33 ± 0.31. These data support the previous research which showed a reduction in egg laying in *C. elegans* mutated to exhibit cardiomyopathy (AlKahleefa, 2020). JM311 fed the HF diet group laid an average of 32.33 ± 0.28 eggs per plate while those fed the HP diet laid 26.00 ± 0.36 eggs per plate. The addition of either high fat or

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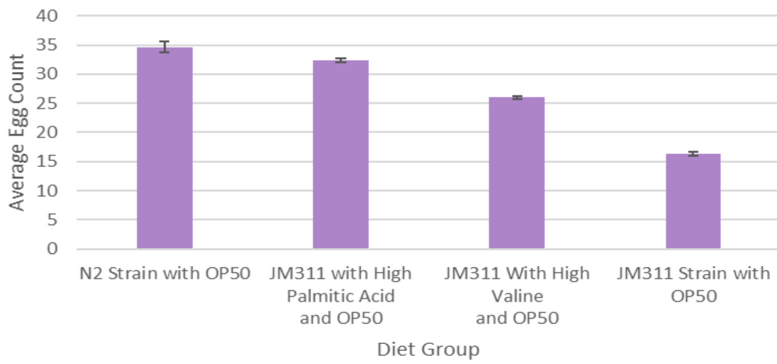

Figure 1. Average egg viability assay egg counts from three trials, for each diet group. Bar graph showing mean eggs laid by each diet group, with standard error bars (n=3). N2 and JM311 were fed the standard control diet of E. coli OP50 and JM311 mutated *C. elegans* were fed the standard diet of *E. coli* OP50 plus either a HF diet or HP diets.

high protein to the diet of JM311 *C. elegans* resulted in more eggs than the JM311 strain fed the standard OP50 diet (Figure 1 and Table 1). These data suggest that both HP and HF diets played a role in improving the LVH conditions.

A Mann-Whitney test was conducted comparing the brood sizes (egg counts) of both experimental groups to each other. Because a small sample size (n=3) was used and there was one outlier present, a non-parametric Mann-Whitney analysis was conducted to determine whether HF diet groups laid significantly more eggs than the HP diet groups (Table 2). With a significance value less than p= 0.05, the number of eggs laid by worms in the HF diet group was significantly larger than that of the HP diet group. This suggests that the HF diet improved the condition of the worms significantly more than the HP diet group, with its median egg count the closest to the N2 wildtype worm's median egg count (Table 1).

Table 1. Average egg viability assay egg counts of all three trials, for each diet group. Table showing mean ± SD eggs laid by each diet group (n=3). Two groups of JM311 worm strains were fed HF diets or HP diets, and one group of JM311 worms and one group of N2 worms were fed typical OP50 bacteria and grown at room temperature.

Diet Group	Average Egg Count for Three Trial (eggs)
N2 Strain with OP50	34.67 ± 0.92
JM311 Strain with OP50	16.33 ± 0.31
JM311 With High Valine and OP50	26.00 ± 0.36
JM311 with High Palmitic Acid and OP50	32.33 ± 0.28

Table 2. Egg count comparison of the JM311 HP diet group and JM311 HF diet group. Table showing Mann-Whitney statistics values (n=3) of median egg counts of HP and HF worms of the egg viability assay. JM311 worms were fed both HP and HF diets. Mann-Whitney, p = 0.0383

Mann-Whitney statistic values
p-value
0.0383

Table 3. Egg count comparison of the JM311 HP diet group and JM311 HF diet group to N2 wildtype worms. Table showing Mann-Whitney statistics value (n=3) comparing the median egg counts of the N2 worms and the HP and HF worms. JM311 worms were fed either a HP or a HF diet, and N2 wild-type worms were fed the standard *E.coli* OP50 diet.

	Mann-Whitney statistic values
Diet Group	p-value
JM311 HF vs N2 Standard	0.65
JM311 HP vs N2 Standard	0.1642

A Mann-Whitney test was conducted comparing the brood sizes (egg counts) of both JM311 experimental groups to the N2 control group which was fed the standard diet of *E.coli* OP50. Because a small sample size (n=3) was used and there was one outlier present, a non-parametric Mann-Whitney analysis was conducted to determine whether the HF diet group or the HP diet group laid a similar number of eggs as compared to the N2 worms (Table 1 and Table 3). With a significance value of 0.05 (95% likely that there is a difference between data values), the number of eggs laid by worms in the HF or HP diets was not statistically different from the N2 control group (p-value of 0.1642 and 0.65 respectively). These data suggest that the HP or HF diets improved the left-ventricular hypertrophy condition to the point that the animals laid similar numbers of eggs as compared to healthy worms.

3. Discussion

When comparing the effect of a high protein diet to a high fat diet (Table 1 + 2) the results show a statistically significant larger egg count for the JM311 worms fed the high fat diet as compared to worms fed the high protein diet, suggesting that high saturated fat consumption may lead to a less severe cardiomyopathy than that of high protein consumption. This means that the original hypothesis that saturated fat diets would improve LVH rates more than the HP diet is supported by the data of this experiment.

However, there was a lack of a significant difference between egg counts of both the high protein diet worms ($p = 0.65$) and the high fat diet worms ($p = 0.1642$), when compared to the N2 wildtype worms (Table 3). This suggests that, in addition to the HF diets, the HP diet also significantly increased egg counts when compared to the N2 wildtype strain. These data suggest that both diets may have the potential to improve LVH conditions.

The results presented in this study partially align with published research. Past studies suggested that high unsaturated fat diets decreased cardiovascular disease risk (Trieu et al., 2021), while high protein diets decrease cardiovascular health (Benian & Epstein, 2011). Though a HF diet improved left-ventricular hypertrophy when compared to the HP diet, both diets improved the LVH as measured by egg laying ability. Thus, it cannot be concluded based on the data presented here that a high protein diet is detrimental to cardiovascular health.

This inconsistency between past research and the research presented here may be attributed to an outlier in the N2 control group. In the future, this study should be conducted again, but with more trials to accommodate for potential outliers. Further, because both nutrients improved the left-ventricular hypertrophy, future research could test whether a combination of both nutrients improves the condition of the worms more than just one nutrient together.

4. Conclusion

C. elegans mutated with left-ventricular hypertrophy were treated with either a high fat diet or a high protein diet and improvement in their egg laying ability was measured as a function of cardiovascular health. The egg viability assay indicated the severity of the condition, where the number of eggs laid correlated with the health of the worms. Using a nonparametric Mann-Withney test and observing the data it was found that HF diets improve conditions of the worms statistically more than the HP diets. In addition, it was found that both HP and HF diets improved LVH conditions in JM311 worms as compared to N2 control worms. This study has identified a potential connection between improvement in left-ventricular hypertrophy with the consumption of both saturated fat and proteins, providing clinicians and patients guidance on what they should be eating.

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