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The Effect of Low-Carbohydrate Intake and Exercise on the Rate of Weight Gain and Blood Glucose Fluctuation in Female Mice

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ABSTRACT

The combination of overeating large amounts of high fat and high carbohydrate foods and lack of exercise is causing an increase in serious health issues for Americans. The question that this research will try and determine is, whether or not the intake of a low-carbohydrate diet affects the weight or blood glucose of female mice? To answer this question one used twenty-four, 21 day-old female mice in two groups, a low-carbohydrate group and a control group. The control group was fed a diet containing 14% protein, 14% fat, and 72% carbohydrates, the low-carbohydrate group was fed 17.3% protein, 21.2% fat, and 48.5% carbohydrates, half of each group was forced to exercise by swimming for four weeks. Weight measurements were taken every week and the blood glucose measurements were taken at the beginning and end of the study. The results obtained from the exercised mice on a low-carbohydrate vs. control diet showed a P-value of 0.066 and one would have only needed 13 mice in each group to prove a statistically significant difference. The P-value for non-exercised mice in the control vs. low-carbohydrate diet was 0.364 showing no significant difference. The data collected from both the non-exercising and exercising mice on a control vs. low-carbohydrate diet shows absolutely no statistically significant difference. There are few studies that have tested the difference in a low-carbohydrate diet vs. a control diet which makes it hard to determine an accurate study set-up, however through this research we have made much progress in determining how to set up a more successful study and how the amount of carbohydrates and exercise can affect female mice.

Keywords: *Low Carbohydrate, Exercise, Non-Exercise, Weight, Blood Glucose, Female Mice*

INTRODUCTION

The combination of overeating foods such as large amounts of carbohydrates and fat rich foods and a lack of exercise is causing many serious health issues for Americans (Hill, Melanson and Wyatt, 2000; 1997; Dourmashkin, et. al., 2005). Although it is known that weight gain is not just determined by the intake of carbohydrates and the lack of exercise, this study will focus on carbohydrates specifically. The objective for this research is to determine how different amounts of carbohydrates consumed and exercise affects weight fluctuation and blood glucose. It has already been shown that weight gain results from too much energy intake and not enough energy output (Strasser, Spreitzer and Haber 2007), meaning that the amount of calories consumed is more than the energy exerted by the body. Knowing this, a low-carbohydrate diet with and without exercise compared to a regular carbohydrate diet with and without exercise will be used to achieve the objective. This is to determine how different amounts of carbohydrates consumed and exercise affects weight fluctuation and blood glucose.

Questions that will be answered by this research are: Can a low intake of carbohydrates be an effective way to achieve weight loss or less weight gain in mice? Will mice that consume a greater amount of a low-carbohydrate diet gain more weight than a mouse that is conservative with food? Is it possible for mice to gain large amounts of weight if they are active?

Based on research done by the National Academies of Science it is known that a group of mice on a low carbohydrate diet will be less likely to gain weight, but it is also know that low-carbohydrate diets usually contain high-fat percentages which can cause weight gain (Foo, et.al., 2008). The control group with a high carbohydrate intake will have different results, according to Clinical Nutrition, obesity can result from excessive fat or carbohydrates intake.

MATERIALS AND METHODS

Animals and Diets

Female, CD-1 21- day-old mice were obtained from Charles River Laboratories. Each mouse was kept in a separate cage and had unlimited access to water and food. The control group of 12 mice ate a normal mouse dietary intake which is high in carbohydrates. The diet contained 14% protein, 14% fat, and 72% carbohydrates. Half of the group was forced to exercise and half did not exercise. The low-carbohydrate group also consisted of 12 mice and was fed a diet containing 15.23% protein, 42% fat and 42.7% carbohydrates, half of this group was forced to exercise and half did not exercise. The mice received the same amount of caloric density (4.5Kcal/g) just in a different percentage rate of carbohydrates, fats, and protein, but their overall caloric intake depends on the amount of food ingested.

Exercise

Half of the mice from both the control and low-carbohydrate group were individually placed into large plastic containers filled with room temperature water, forcing the mice to swim and expend energy. The exercising mice underwent ten minutes of swimming three days a week (week one), after the first week the exercise time was increased to fifteen minutes a day three days a week (week two), after the second week the exercise time decreased to ten minutes but the days they were forced to exercise increased to four days a week (week three), in the last week the exercise time was increased again to fifteen minutes and stayed at four days a week (week four).

Measurement of Weight and Blood Glucose Level

All mice received a label so that the data from their weight and blood glucose levels could be recorded accurately. The control group was assigned the label 1-C through 12-C and the low carbohydrate group was assigned the label 1-LC through 12-LC. All twenty-four mice were weighed at the beginning of the four week study and weighed once a week until the study was complete. To measure the individual mouse weight, each mouse was individually placed in a metal container and put on the scale to be weighed. The weights were measured to 0.01g to ensure accuracy. The metal container was used so the mouse would not escape during the weighing process.

Each mouse's blood glucose was taken at the beginning of the study after a four hour fasting period in order to get an accurate reading and then at the end of the four week study. A normal blood glucose reading for mice is between 62mg/dl and 175g/dl after a four hour fasting period (Colemann, et.al. 2007). The blood glucose readings were taken using a blood glucose monitoring system. Each mouse's tail was cleaned with alcohol and then snipped with sanitized scissors, the blood produced was placed on the blood glucose testing strip The mouse's tail was then cleaned again with alcohol to ensure their safety and eliminate any chance of infection.

RESULTS

Data was obtained from twenty-four female mice being tested for a difference in weight and blood glucose. The goal for the statistical analysis is to determine whether or not there is a significant relationship between the differences in both weight blood glucose fluctuation, carbohydrate intake and exercise level. This raw data was transferred to Sigma Plot where a paired t-test was run for both weight gain and blood glucose fluctuation. When running a paired t-test it is assumed that the data was obtained by randomized, unbiased study design and the sampling distribution is normal.

The paired t-test was run to determine the difference in weight gain between subjects on a low carbohydrate diet and a normal carbohydrate diet (control group) as well as between female mice that were exercised and non-exercised. Since the data from the control vs. low-carbohydrate exercised female mice violated the normality assumptions of the t-test, the Mann-Whitney U-test was used. It was used because it is a nonparametric test of significance that examines whether the data values in one sample tend to be larger than the data values in a second sample. The results in Table 1. shows that the P-value was 0.066 which is only 0.016 greater then 0.05 which suggests that there could be a possible significant difference. To see how many mice would have been needed to achieve a significant difference, a sample size t-test was run. The results for the sample size t-test shown in Table 2. show that 13 mice would have been needed to give the data a statistically significant difference.

Table 1. T-test/Mann-Whitney U Test: Shows the median and the 25% and 75% percentiles for the data, it also shows that the P-value is estimated at 0.066 which is close to 0.05. This suggests that there is a possibility for a significant difference.

Control vs. Low-Carbohydrate Exercised Female Mice (Weight)

Group	6	Median	25%	75%
C Change (g)	6	7.97	7.05	9.53
LC Change (g)	6	4.395	2.1	4.58

Mann-Whitney U Statistic= 6.000
T = 51.000 n(small)= 6 n(big)= 6
P(est.)= 0.066 P(exact)= 0.065

Table 2. Sample Size T-test: The sample size t-test shows that 13 mice would have been needed to show a statistically significant difference in weight for exercised female mice.

Control vs. Low-Carbohydrate Exercised Female Mice (Weight)	
Sample Size	13
Difference in Means	3.212
Standard Deviation	2.8
Power	0.8
Alpha	0.05

When the data obtained for the control vs. low-carbohydrate non-exercised female mice was tested through the t-test it passed both the normality and equal variance tests. The difference in the mean values (3.118) of the two groups was not great enough to reject the possibility that the difference was due to random sampling variability. Shown in Table 3. the P-value was equal to 0.364 which is much greater then 0.05. This size of a P-value shows that there is no relationship between the type of diet consumed by the female mice and the weight gained in non-exercised female mice.

Table 3. Paired T-test: The data put through the T-test passed the normality and equal variance test. The difference between the mean values for the control and low carbohydrate diet in non-exercised female mice (3.118) is not great enough to reject the possibility that the difference is due to random variation. It also shows that because the P-value is 0.364 there is no statistical significant difference between the two groups.

Control vs. Low-Carbohydrate Non-Exercised Female Mice (Weight)

Normality	Passed	(P = 0.102)		
Equal Variance	Passed	(P = 0.449)		
Name	N	Mean	StDev	SEM
C-Change (g)	6	6.787	4.991	2.037
LC-Change (g)	6	9.905	6.291	2.568
Difference	-3.118			
t = -0.951 with 10 degrees of freedom.				
(P = 0.364)				

Although the paired t-test showed no significant relationship between the type of diet consumed and weight gained in non-exercised female mice, a power of the performed test was done with alpha equaling 0.05. The power of the performed test (0.05) is below the desired power of 0.800. This indicates that it is less likely to detect a difference when one actually exists. Table 4. shows that the sample size needed to produce a power of 0.800 and prove that there is a statistically significant difference in weight gain and the amount of carbohydrates consumed by non-exercised female mice is 53.

Table 4. Sample Size T-test: Shows that the sample size need to produce a power of 0.800 and prove that there is a statistically significant difference between weight gain and amounts of carbohydrates consumed is 53 mice.

Control vs. Low-Carbohydrate Non-Exercised Female Mice (Weight)

Sample Size	53
Difference in Means	3.118
Standard Deviation	5.641
Power	0.8
Alpha	0.05

A box plot was used to compare the difference in weight between female mice that exercised but were fed a low-carbohydrate diet and a control diet, as well as non-exercising female mice that were fed a low-carbohydrate diet and a control diet. As one can see in Figure 1. there is a slight significant difference between the female mice that were forced to exercise and fed two different diets. It also shows that there is absolutely no statistically significant difference in the non-exercising female mice that were fed diets containing two different amounts of carbohydrates. It is also apparent that there is no statistically significant difference between the exercised and non-exercised female mice in either the low-carbohydrate or control diet.

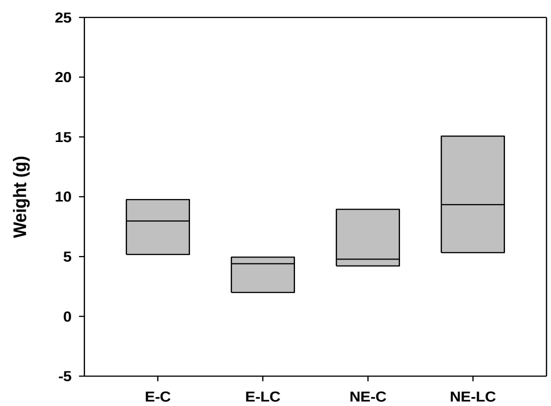

Figure 1. Box Plot: Shows that there is no significant difference between the amount of weight gained for the non-exercised female mice and diet being fed to the female mice. However there is a slight difference in the exercised female mice being fed a low-carbohydrate and a control diet. Although the box plot may suggest that there could possibly be a difference, this difference cannot be determined statistically significant because the P-value is greater than 0.05.

When a paired t-test was run to determine the difference (8.5mg/dl) in blood glucose between the low carbohydrate and a normal carbohydrate diet (control group) in exercised female mice it passed both the normality and equal variance tests, but the difference in the mean values of the two groups was not great enough to reject the possibility that the difference is due to random sampling variability. Shown in Table 5. the P-value was equal to 0.788 which was much greater than 0.05. This shows that there is not a statistically significant difference between the type of diet eaten and blood glucose levels in exercised female mice. A sample t-test shown in Table 6. was then run for non-exercised female mice to determine that 618 mice would have been needed to find any statistically significant difference between the blood glucose fluctuations in exercised female mice on a low-carbohydrate vs. control diet.

Table 5. Paired T-test: Shows that the data passed the normality and equal variance test, and that the difference between the mean values for the low carbohydrate diet (control group) (8.5mg/dl) is not great enough to reject the possibility that the difference is due to random variation. It also shows that because the P-value is 0.788 there is no statistical significant difference between the two groups.

Control vs. Low-Carbohydrate Exercised Female Mice (Blood Glucose)				
t-test				
Normality Test:	Passed	(P = 0.702)		
Equal Variance Test:	Passed	(P = 0.759)		
Group Name	N	Mean	Std Dev	SEM
C-Change (mg/dl)	6	-69	53.844	22
LC-Change (mg/dl)	6	-77.5	52.732	21.5
Difference	8.5 mg/dl			
t = 0.276 with 10 degrees of freedom.				
(P = 0.788)				

Table 6. Sample size test: Shows that 618 mice would be needed to show any statistically significant difference between exercised female mice being fed a control vs. low-carbohydrate diet.

Control vs. Low-Carbohydrate Exercised Female Mice (Blood Glucose)	
Sample Size	618
Difference in Means	8.5
Standard Deviation	53.288
Power	0.8
Alpha	0.05

A paired t-test was then run to determine the difference (11.167mg/dl) in blood glucose for a low-carbohydrate and a normal carbohydrate diet (control group) in non-exercised female mice. The data passed both the normality and equal variance tests, but the difference in the mean values of the two groups was not great enough to reject the possibility that the difference was due to random sampling variability. Shown in Table 7. the P-value was equal to 0.635, which is much greater than 0.05. This shows that there is not a statistically significant difference between the type of diet eaten and blood glucose levels in non-exercised female mice. Since the paired t-test showed no statistically significant difference between the type of diet eaten and blood glucose, a power of the performed test was done for non-exercised female mice with alpha equaling 0.05. Table 8 shows that 194 mice would have been needed to show a statistically significant difference in blood glucose for exercised female mice on a control vs. low-carbohydrate diet.

Table 7. Paired T-test: Shows that the data passed the normality and equal variance test, and that the difference between the mean values for the low carbohydrate and low carbohydrate diet (control group) (11.167mg/dl) was not great enough to reject the possibility that the difference is due to random variation. It also shows that because the P-value is 0.635 there is no statistical significant difference between the two groups.

Control vs. Low-Carbohydrate Non-Exercised Female Mice (Blood Glucose)				
t-test				
Normality Test:	Passed	(P = 0.469)		
Equal Variance Test:	Passed	(P = 0.493)		
Group Name	N	Mean	Std Dev	SEM
C-Change (mg/dl)	6	-24.7	44.818	18.3
LC-Change (mg/dl)	6	-35.8	33.481	13.7
Difference	11.167			
t = 0.489 with 10 degrees of freedom. (P = 0.635)				

Table 8. Sample size test: Shows that 194 mice would be needed to show any statistically significant difference between non-exercised female mice on a

control or low-carbohydrate diet.

Control vs. Low-Carbohydrate Non-Exercised Female Mice (Blood Glucose)	
Sample Size	194
Difference in Means	11.166
Standard Deviation	39.15
Power	0.8
Alpha	0.05

A box plot was used to compare the difference between blood glucose in exercising female mice and non-exercising female mice that were fed a low-carbohydrate diet compared to a control diet. As one can see in Figure 2. there is no statistically significant difference between the mice that did not exercise and the mice that were forced to exercise, in neither the low-carbohydrate or control diet.

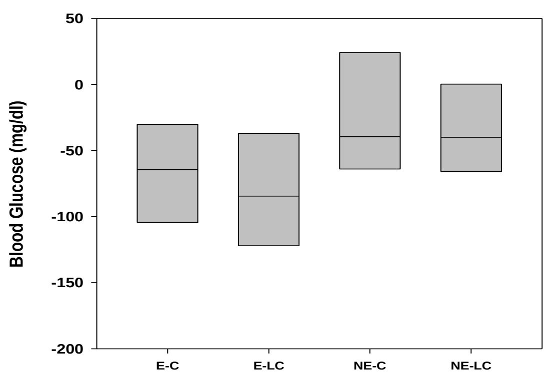

Figure 2. Box Plot: Show that there is no significant difference between blood glucose levels for either exercised or non-exercised mice that have been fed a control vs. low-carbohydrate diet.

DISCUSSION

The objective of this research was to determine how different ratios of carbohydrates consumed and exercise affects weight and blood glucose fluctuation. As shown in the results three out of the four groups that were tested did not show a statistically significant difference. Although there is still evidence that there could be a statistically significant difference between weight and blood glucose and the amounts of carbohydrates consumed by female mice that did and did not exercise, there were not enough mice available to be tested in each group. The control vs. low-carbohydrate exercised female mice (Weight) group on the other hand showed a P-value of 0.066 which is technically not statistically significant but is very close to 0.05 which is considered statistically significant. After reviewing these results I believe there is a way to achieve statistically significant results for the control vs. low-carbohydrate exercised female mice (Weight) group by adjusting the experimental set-up.

If one was to continue this research there are a few things I would suggest changing in the experimental design. The first change that I suggest changing is to test only two groups instead of four groups and only testing the weight change in these mice. This will allow for a greater number of test subjects in each group which will in turn give a greater significance in the results. Next, the diet fed to the female mice should be modified so that there is a greater percentage of carbohydrates and protein and less fat. This will allow the weight gain to be a result of too many carbohydrates and not because to an excess of fat intake. All mice should also be kept in the exact same environment. During the data collection the mice seemed to be sensitive to the environment they were kept in and responded differently. The mice that were kept in a metal cage with no bedding became very agitated and tended to consume more food, but the mice kept in a plastic cage with bedding seemed to be content and did not consume as much food. This could have had an effect on the statistical outcome of my results. The final suggestion to modifying the experimental set-up is to keep track of how much each mouse eats per day. The reason I suggest doing this is because some mice may eat more than others causing the data to be compromised. For example if two mice are consuming the same amount of carbohydrates but one is eating a small amount of food that is high in carbohydrate and another mouse is consuming a large amount of food that is low in carbohydrates, it is not going to have the same affect on the weight of the mouse.

Although the results that were obtained through this research were not what were expected and many of the questions asked were not answered there is now a base for future researchers. With the right experimental set up and execution a future researcher will be able to receive a statistically significant difference in the amount of carbohydrate intake and weight loss in female mice that are exercised and non-exercised.

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