Examining the Effects of Exercise on Human Endorphin Levels Through Hypothesis Testing

Nikhil Sharma, Raghava Govil; Statistics 101B, Lecture 2, Group 16

nikhilsh98@gmail.com

raghava.govil98@gmail.com

Abstract

Stress and anxiety are among the most pressing mental health problems today, especially for college students. Medical research into this area has shown that endorphin hormones released by the pituitary gland into the body help relieve stress, pain, anxiety, and create a general feeling of happiness in humans. Consequently, we decided to study stimulants of endorphin secretion in humans and hypothesized that exercise has an effect on this secretion, as derived from prior literature and research. We decided to subject our participants to the treatments of 100-meter outdoor run, 1-kilometer outdoor run, 5-kilometer outdoor run, 50-meter swim and 200-meter swim. The design used was a 5x5 Randomized Latin Square, with blocks on person-to-person variability and order of treatment. The Latin Squares were repeated 5 times to increase power and error degrees of freedom. Participants chosen were male, aged between 18 to 30, had no health ailments and lived in the city of Macondo. After creating linear models and ANOVA tables in R for each of the Latin Squares, we found exercise to be significant in causing an increase in endorphin level, thus proving our hypothesis. Further analysis and hypothesis testing showed that the longer the duration of the exercise, higher the endorphins increase. Thus, our study concluded that intense and long duration exercises can help reduce stress, pain and anxiety by inducing a steep increase in endorphin levels.

Introduction

Endorphins are hormones released by the pituitary gland into the bloodstream or propelled into the brain. The secretion of this hormone is associated with a general feeling of euphoria, as it plays a role in the reward system of the brain. In other words, endorphins create a relaxed psychological state, which is believed to reduce mental stress and pain in humans. Due to this positive effect of the release of endorphins, the study of the stimulus of endorphins has been a common and popular topic of research for scientists ever since its discovery. As per research by Haynes IV et al., there might be a relationship between exercise and the increase of endorphin levels in humans, but due to the large amount of person-to-person variability, it has been a complex task to find a concrete relationship between exercise and blood endorphin level.

A common study that has been conducted is the effect of different types of exercise on blood endorphins (Kraemer). Types of exercise include resistant and non-resistant. Resistant exercises are those which require the person doing exercise to overcome some resistant force, such as water in swimming. Non-resistant exercises, such as running, do not require the person to overcome a resistant force.

Since there are a host of positive impacts associated with endorphin release, we felt that research into the topic would be worthwhile, since intense mental stress afflicts many fellow students. We studied the effects of different types of exercise on the increase in blood endorphin levels in humans. We subjected our participants to the following exercises: 100-meter outdoor run, 1-kilometer outdoor run, 5-kilometer outdoor run, 50-meter swim freestyle and 200-meter swim freestyle. Resistant exercises include the swimming exercises, while non-resistant exercises include the running exercises. To further enhance our research, we decided to test out two more simple hypotheses, based on the results from our main hypothesis test regarding exercise and endorphins. We tested whether there was a relationship between time taken to finish exercise and endorphin increase as well as whether resistant and non-resistant exercises had a difference in impact on endorphin increase.

<u>Methodology</u>

Participants

Our participants were chosen from the virtual *Island* provided to us. Prior literature suggests that one significant source of variation in endorphin release after exercise is stage of menstrual cycle (Goldfarb et al.). Consequently, in order to minimize bias, we decided to choose only males since it is not possible to check if a female is menstruating or not on the *Island*. These males were chosen in the athletic age group of 18-30 years and did not have any health ailments like asthma or diabetes, since these factors could potentially affect athletic performance. This was done in accordance to medical research stating that endorphins level decreased with age (Gambert et al.). We also picked males from the same city, Macondo, from the *Island* to reduce variability. We picked 25 subjects with the above constraints. We could not control the factors of athletic ability and recovery period for each subject. Since we expected these factors to introduce high person-to-person variability, we chose subjects under the listed constraints to minimize variance from other sources. The process of collecting this sample included going from house to house on the Island, filtering out potential candidates with the above constraints and then obtaining their consent to participate in the study. Since the *Island* does not allow for random selection, this part of the study was single-blinded.

Design

For our study on the effects of different types of exercise on endorphin increase, the Randomized Latin Square design fits our needs. This design allows for blocking on two nuisance factors. For our experiment, as literature indicated, the two nuisance factors were person-to-person variability and order of treatment. Controlling for these two nuisance factors allowed us to reduce the variability and error in our results. We used a 5x5 Latin Squares design, due to the five treatments that we had: 10-meter outdoor run, 1-kilometer outdoor run, 5-kilometer outdoor run, 50-meter swim freestyle and 200-meter swim freestyle. In order to increase our power and error degrees of freedom, we decided to repeat our Latin Squares design 5 times. Each participant represented a row and the columns were represented by the order of treatment. Each treatment was assigned a letter from A to E respectively, which resulted in the following Latin Square matrix, designed using the function rlatin() in the R package *magic*:

A	B	E	D	C
C	A	D	B	E
B	C	A	E	D
D	E	C	A	B
E	D	B	C	A

Once the above matrix was made, we used the built-in random number generators in R to randomly assign the order of treatment to each participant, thus obtaining a single-blinded experiment.

The equation of our effects model is as follows:

$$y_{ijk} = \mu + \alpha_i + \tau_j + \beta_k + \varepsilon_{ijk} \begin{cases} i = 1, 2, \ldots, p \\ j = 1, 2, \ldots, p \\ k = 1, 2, \ldots, p \end{cases}$$

Here, y is the endorphin level, μ is overall mean, α is the row effect, β is the column effect, τ is the treatment effect and ε is the random error. Our null hypothesis is that the row, column and treatment effects are 0, while the alternative hypothesis is that at least one effect is not 0. We used linear models in R to fit the effects models for each of the 5 Latin Squares. We also combined the data from the 5 Latin Squares and fit an effects model for the aggregated data.

With the combined data, we tested our other two hypotheses. To test whether there was a relationship between time taken to finish exercise and endorphin increase, we fit a linear model in R. To test whether resistant and non-resistant exercises had a difference in impact on endorphin increase, we implemented two sample t-tests in R.

Procedure and Instruments

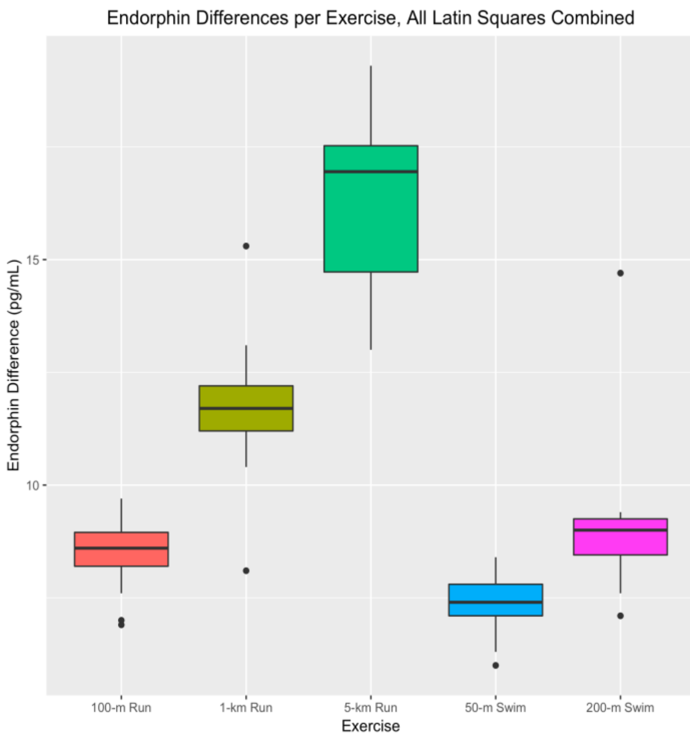
Our data collection process took about 3 days. We used the *Island* tool provided to us to collect the data we needed from the virtual participants. Each participant was randomly assigned the five exercises mentioned above in accordance to the randomly generated matrix. We chose how to randomize the exercises per subject by assigning each participant and each treatment to vector objects in R and using its built-in sampling functions.

The endorphin levels per participant were calculated in picograms per milliliter (pg/mL) right before the exercise. Research done by Goldfarb and Jamurtas indicates that the maximum endorphin spike occurs about one minute after exercise is completed. Thus, after each participant finished their exercise, we waited one minute before re-measuring their endorphin levels. In order to make sure that the treatments were independent from each other, we decided to give each participant 3 hours of recovery, considering that all of our participants were young and athletic and did not have any health ailments. The data collected every five hours were entered into an Excel spreadsheet which was then uploaded to R to carry out the linear and ANOVA modeling and t-tests.

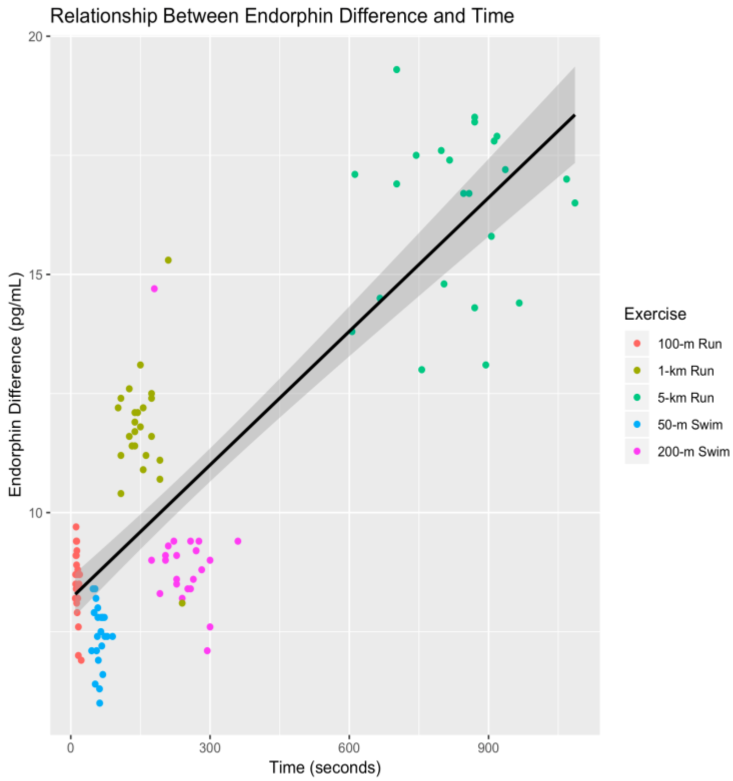
Data Analysis and Results

Exploratory Analysis

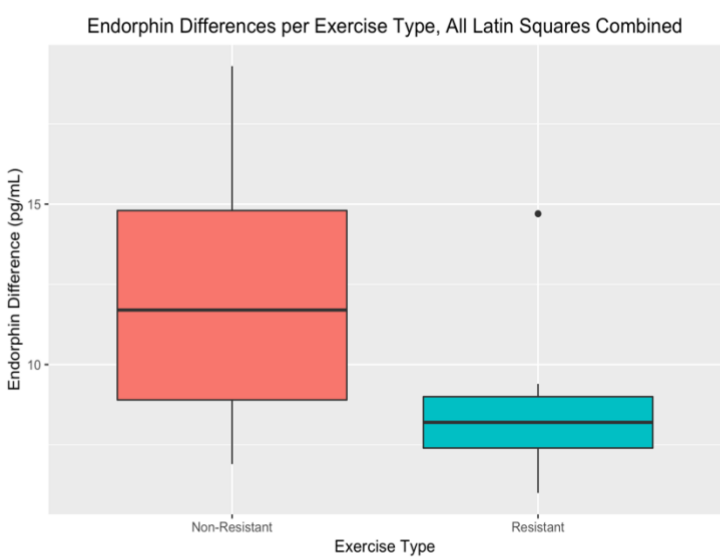
Boxplots for Each Latin Square and Combined Latin Square:



Relationship Between Endorphin Difference and Time Taken to Complete Exercise:



Boxplot for Comparing Exercise Types:



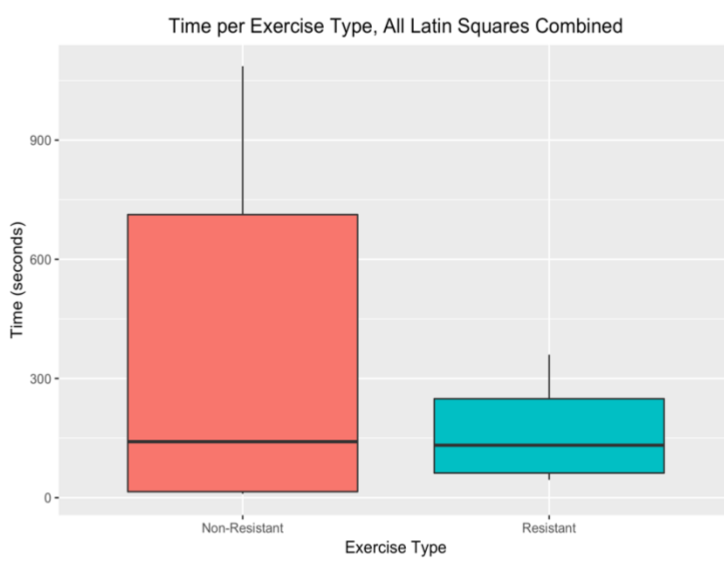
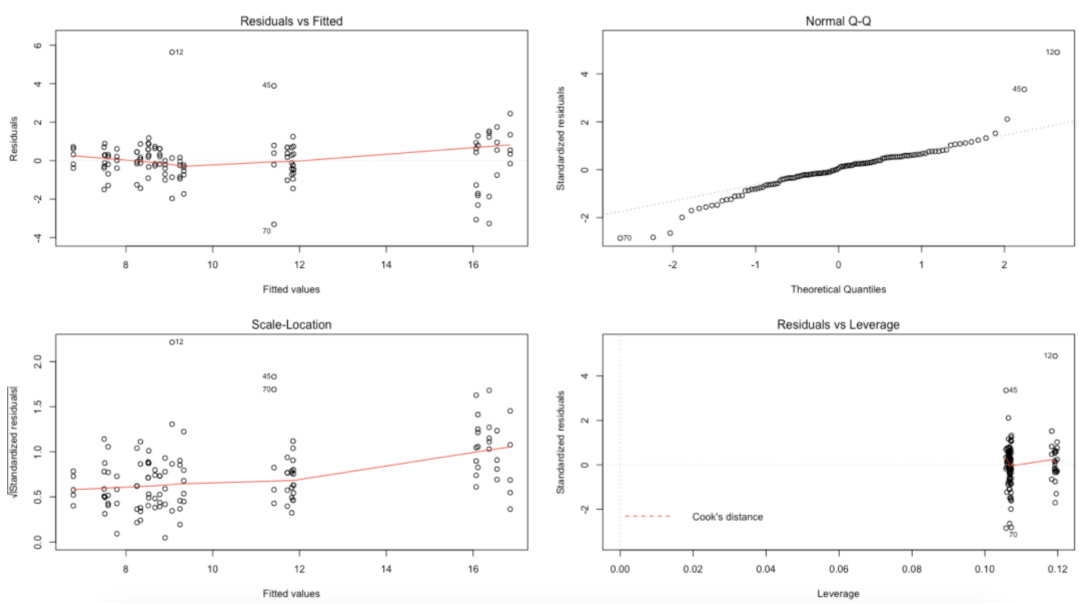


Table: Comparing Exercises, Combined Latin Square

Exercise	Type of Exercise	Mean of Endorphin Difference (pg/mL)	Mean of Time (seconds)
100-m Run	Non-Resistant	8.504	13.733
1-km Run	Non-Resistant	11.732	150.720
5-km Run	Non-Resistant	16.383	835.043
50-m Swim	Resistant	7.417	62.243
200-m Swim	Resistant	9.030	247.565

Adequacy of Linear Models

We fit linear models in R for each of the 5 Latin Squares. From each of the 5 Latin Squares, we found 2, 1, 0, 1, and 2 bad leverage points respectively, for a total of 6 bad leverage points out of 125 observations. We then combined the data from all of the Latin Squares and fit a linear model for the combined data, without the bad leverage points. Its diagnostic plots are as follows:



The key assumptions for a linear model hold here; there was presence of a linear pattern, the residuals were normally distributed, the residuals had constant variance and there were no bad leverage points.

ANOVA Tables

After establishing the adequacy of the linear models for each Latin Square and the combined Latin Square data, we used the anova() function in R to obtain the ANOVA tables for each Latin Square. Each ANOVA table gives Degrees of Freedom, Sum of Squares, Mean Sum of Squares, F-value and the corresponding *p*-value. Based on our significance level of 5% ($\alpha = 0.05$), we rejected the null hypothesis that a variable was significant, if our *p*-value was less than 0.05. As seen in the tables below, all 5 Latin Squares showed that the factors (i.e., the exercise) were significant. However, we did not see any significance for the person to person variability (i.e., the row). Latin Squares 1 and 5 showed significance for the order of treatment (i.e. the column).

ANOVA: Latin Square 1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Row	4	17.903	4.4758	1.3332	0.323201
Column	4	48.463	12.1157	3.6090	0.045381*
Exercise	4	125.221	31.3052	9.3250	0.002088*
Residuals	10	33.571	3.3571		

ANOVA: Latin Square 2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Row	4	1.652	0.413	0.1548	0.9568
Column	4	11.062	2.766	1.0364	0.4315
Exercise	4	233.873	58.468	21.9111	3.389e-05*
Residuals	11	29.353	2.668		

ANOVA: Latin Square 3

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Row	4	7.718	1.929	0.9276	0.4800
Column	4	3.162	0.790	0.3800	0.8187
Exercise	4	259.190	64.797	31.1516	2.974e-06*
Residuals	12	24.961	2.080		

ANOVA: Latin Square 4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Row	4	2.686	0.671	1.0961	0.4057
Column	4	8.002	2.000	3.2656	0.0538
Exercise	4	275.267	68.817	112.3344	7.67e-09*
Residuals	11	6.739	0.613		

ANOVA: Latin Square 5

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Row	4	3.239	0.810	1.8038	0.20472
Column	4	8.710	2.178	4.8514	0.01956*
Exercise	4	290.869	72.717	162.0099	4.801e-09*
Residuals	10	4.488	0.449		

ANOVA: Combined Latin Squares

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Row	4	9.69	2.423	1.6121	0.1766
Column	4	4.87	1.217	0.8099	0.5215
Exercise	4	1223.13	305.781	203.4327	<2e-16*
Residuals	106	159.33	1.503		

* *indicates a p-value < 0.05*

Discussion

After collecting our data in different Excel spreadsheets for each Latin Square, we loaded the data into R. Since we recorded endorphins level before and after exercise, we created a new column in each dataset for the difference in endorphin levels (after - before). Our linear models regressed endorphin difference on row (person-to-person variability), column (order of treatment) and exercise. Based on diagnostic plots for the linear models for each Latin Square, we noticed deviation from adequacy due to the presence of bad leverage points. Based on the Cook's Distance and Standardized Residuals of each prediction from the linear model objects, we took a closer look at points whose Cook's Distance exceeded $\frac{4}{n-p-1}$ and whose absolute value of Standardized Residual exceeded 2. Since all of the data was virtual, the margin for error was higher than that of more traditionally collected data. Thus, after examining each of our bad leverage points, we saw that they deviated greatly from the rest of the data and decided to take them out in the interest of model adequacy.

Once all the bad leverage points were removed, and our diagnostic conditions were satisfied, we used the anova() command in R to obtain the ANOVA tables for each of the Latin Square, as shown above. Under our α of 0.05, all our Latin Squares showed the exercise to be significant. Latin Squares 1 and 5 even had the order of treatment (i.e., the columns) to be significant. Our main point of interest was the combined Latin Square due to its high power and larger error degrees of freedom. As hypothesized, the ANOVA table for this combined Latin Square also showed exercise to be significant.

After establishing that there was an effect of exercise on endorphin levels, we wanted to find which exercise seemed to have the maximum impact on endorphin increase. For this, we plotted boxplots for each Latin Square as well as the combined Latin Square, which have been shown above. We can clearly see that the 5-kilometer run and the 200-meter swim, the two exercises that took the longest to finish, increased the endorphin levels the most. We further studied the relationship between time and endorphin difference by fitting a linear model that regressed endorphin level on time of exercise in seconds. After transforming the y-variable of endorphin difference through an inverse response plot, we got the following model:

$$(Endorphin\ Difference)^3 = 394.5232 + 4.6528(Time)$$

This model had an R^2 of 0.7257, and all assumptions of a linear model were satisfied: there was an existence of linear pattern, the residuals were normally distributed, the residuals had constant variance, and there were no bad leverage points.

We also wanted to examine the difference in endorphin release between our resistant swimming exercises and our non-resistant running exercise. To analyze this, we did a two-sample t-test, where the null hypothesis was that the means in endorphin difference for resistant and non-resistant exercises were the same, and the alternative hypothesis was that they were not the same. The mean endorphin increase for resistant exercises was 8.224 pg/mL, while the mean endorphin increase for non-resistant exercises was 12.2 pg/mL. Our p-value for the t-test was 4.097×10^{-14}; assuming a confidence level of 0.05, we rejected the null hypothesis, and accepted the alternative hypothesis that the means of endorphin difference for resistant and non-resistant exercises were different. We did another two-sample t-test where the null hypothesis was that the means in time for resistant and non-resistant exercises were the same, and the alternative hypothesis was that they were not the same. The mean time for resistant exercises was 154.9043 seconds while the mean time for non-resistant exercises was 323.6611 seconds. Our p-value for the t-test was 0.0003573; assuming a confidence level of 0.05, we rejected the null hypothesis, and accepted the alternative hypothesis that the means of time for resistant and non-resistant exercises were different.

Conclusion

Our primary objective was to study the effects of different types of exercise on the increase in blood endorphin levels in humans. Since endorphins help reduce stress, anxiety and pain, the results can prove very useful to people with high stress levels.

Our first hypothesis proved that there is a relationship between exercise and endorphins, with exercise in general inducing an increase in endorphin secretion. The five exercises increased endorphin levels in descending order as follows:

1. 5-kilometer run
2. 1-kilometer run
3. 200-meter swim
4. 100-meter run
5. 50-meter swim

Based off of these results, we tested our two other hypotheses, which showed that longer the exercise, the greater the increase in endorphin levels and that there is a difference in impact between resistant and non-resistant exercises on endorphins.

Our statistically valid model that regressed endorphin increase on time showed that there is a relatively strong relationship between the two through an R^2 of 0.7257. A possible explanation for this might be that as one has more time to do exercise, they have more time to clear their minds and thus become less mentally stressed as they exercise more. Thus, people might want to exercise for longer periods of time if they would like to reduce more stress.

This conclusion also has important implications for researchers. In a scenario where measuring endorphin levels is not feasible or too expensive, a researcher could measure time of exercise and reasonably estimate endorphin difference given the high R^2 value and validity of the regression.

We also found that there was a statistically significant difference between the means of endorphin increase for resistant and non-resistant exercises. The mean endorphin increase for non-resistant exercises was higher than the mean endorphin increase for resistant exercises. However, this is likely swayed by the fact that there was a significant difference between times of resistant and non-resistant exercises, as given by a two-sample t-test. As we saw in our linear model, time is a strong predictor of endorphin increase; thus, time likely contributes to the difference in endorphin increase between resistant and non-resistant exercises. To improve this specific hypothesis test in the future, we could block on time of exercise to mitigate its influence on the response of endorphin increase.

In addition to blocking on time for our hypothesis test on the impact between resistant and non-resistant exercises on endorphins, there are many other potential improvements and further studies for our analysis. Another key improvement would be to select a truly random sample from the *Island*, using a web-scraping script. A possible future study could be to monitor endorphin levels during exercise. Then, we could compare endorphin levels before, during and after exercise rather than simply before and after. Finally, we could examine the tradeoff between physical fatigue and alleviation of mental stress based on time of exercise. We saw in our studies that longer exercise periods tend to result in a higher endorphin increase; however, eventually, if one exercises too long, physical fatigue might overtake these positive effects, so this might be a topic worth analyzing.

Overall, based on our studies, we recommend that highly stressed individuals engage in slightly intense exercises such as 5-kilometer runs so that they can see a boost in endorphin levels and thus a lower level of stress.

Sources

Goldfarb, Allan H., and Athanasios Z. Jamurtas. "β-Endorphin response to exercise." *Sports Medicine 24.1 (1997): 8-16.*

Kraemer, WILLIAM J., et al. "Effects of different heavy-resistance exercise protocols on plasma beta-endorphin concentrations." *Journal of Applied Physiology 74.1 (1993): 450-459.*

Andrea, Leuenberger. "Endorphins, exercise, and addictions: A review of exercise dependence." *Journal for Undergraduate Publications in the Neurosciences 55.3 (2006): 291-297.*

Haynes IV, James T., et al. "Experimental effects of acute exercise on episodic memory function: considerations for the timing of exercise." *Psychological reports* (2018): 0033294118786688.

THOMPSON, Valerie A., and Jamie ID CAMPBELL. "A power struggle: Between-vs. within-subjects designs in deductive reasoning research." *Psychologia* 47.4 (2004): 277-296.

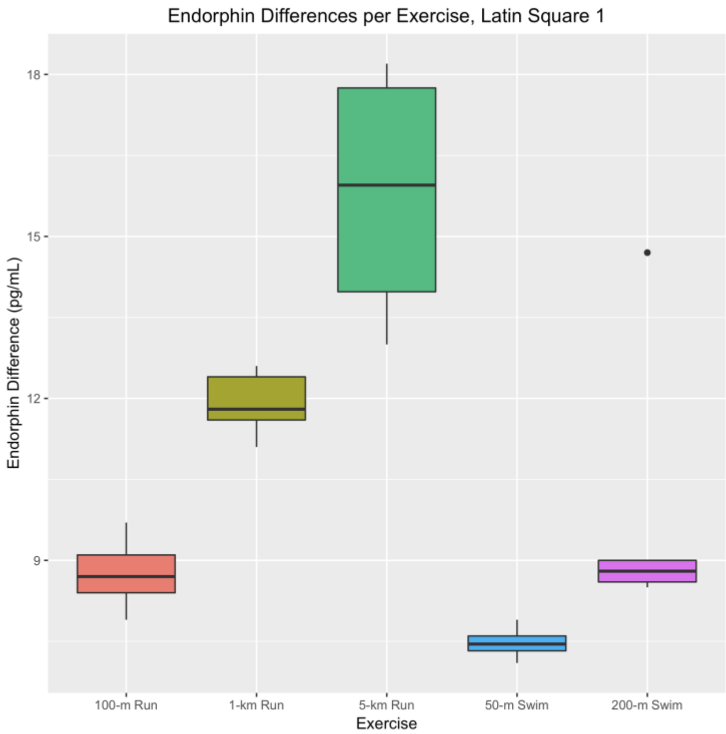
Department of Health & Human Services. "Resistance Training – Health Benefits." *Better Health Channel*, Department of Health & Human Services, 28 Feb. 2014, www.betterhealth.vic.gov.au/health/healthyliving/resistance-training-health-benefits.

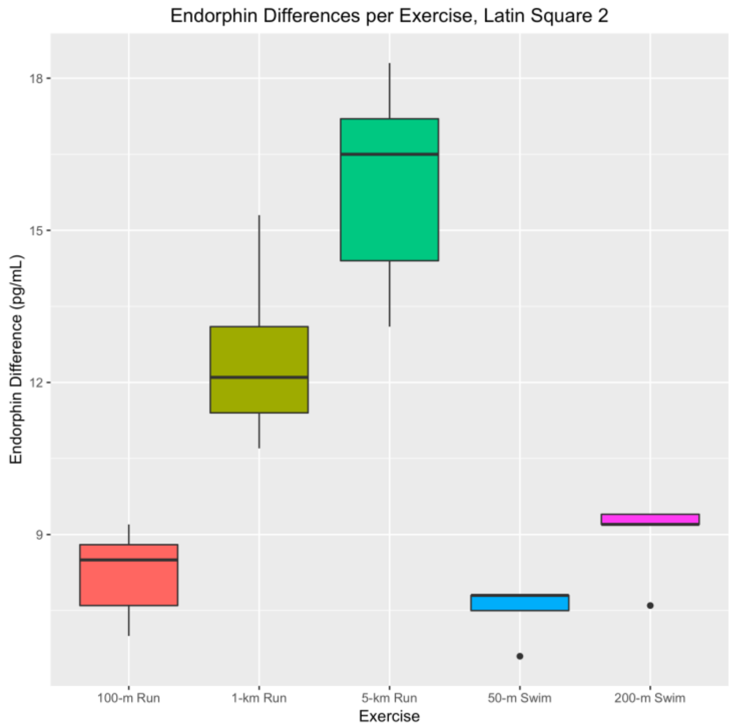
Goldfarb, Allan H., et al. "Gender effect on beta-endorphin response to exercise." *Medicine and science in sports and exercise* 30.12 (1998): 1672-1676.

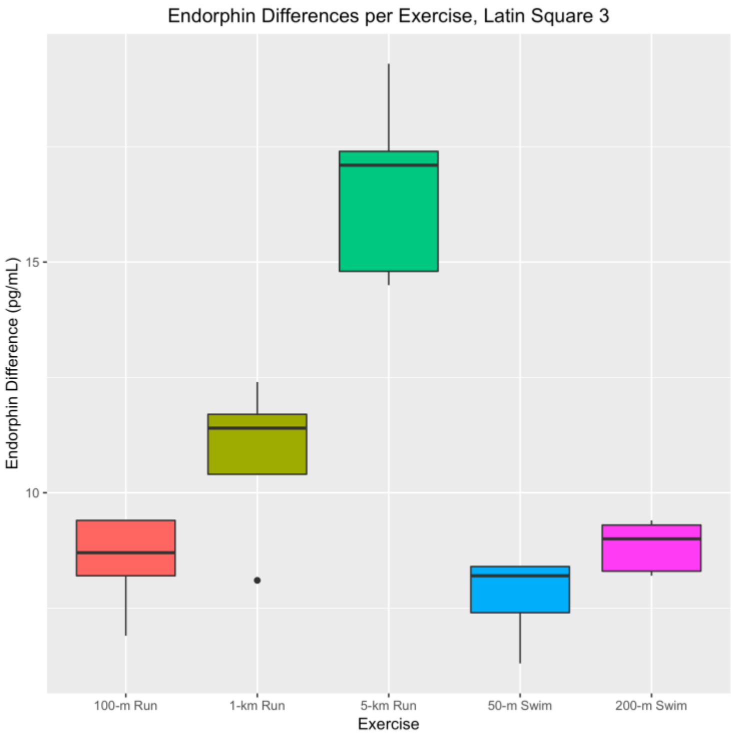
Gambert, S. R., et al. "Age-related changes in central nervous system beta-endorphin and ACTH." *Neuroendocrinology* 31.4 (1980): 252-255.

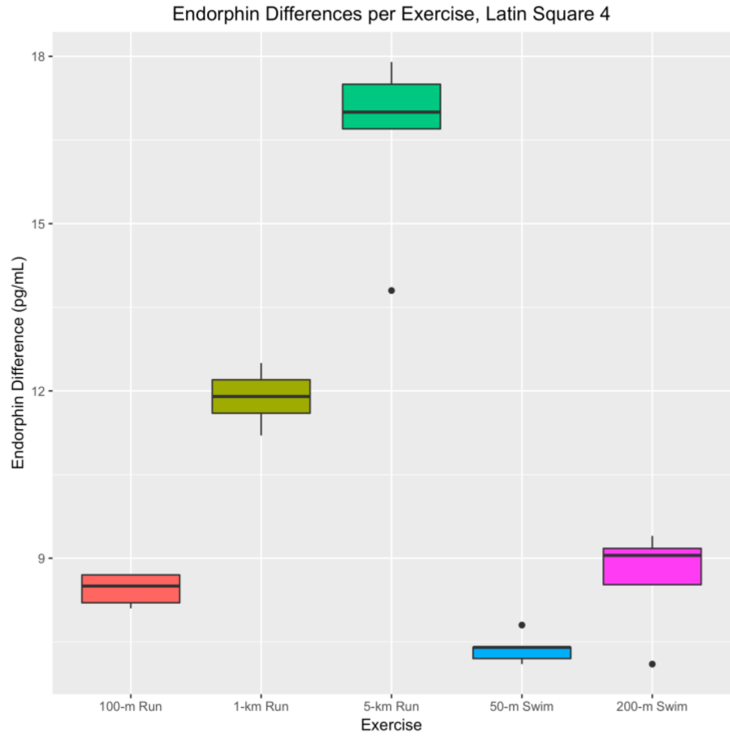
Appendix

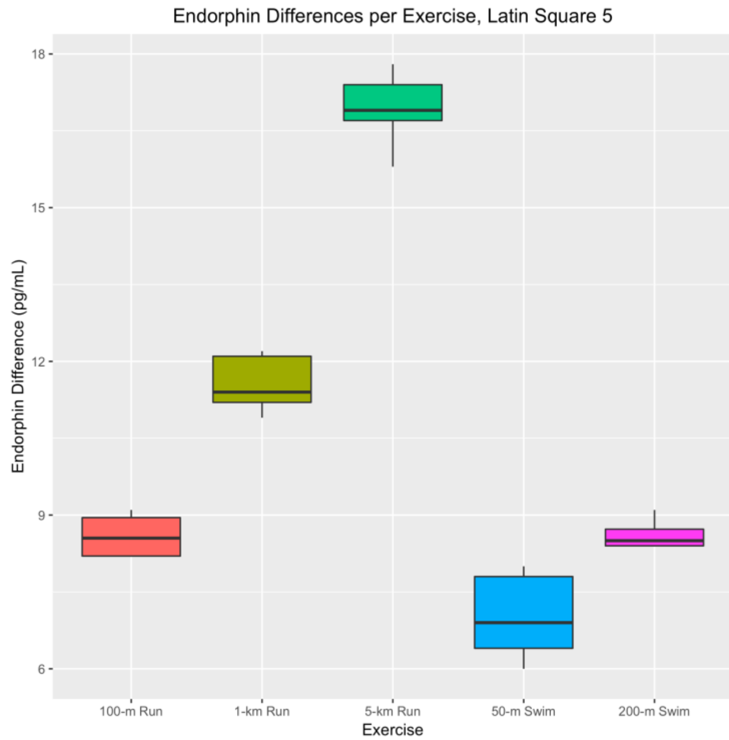
Boxplots for individual Latin Squares:











Diagnostic Plots for the Time-Endorphin Level Linear Model: