The Impacts of Exercise and Age Groups on Blood Melatonin Levels

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Abstract:

Studies have found conflicting evidence regarding the impact of exercise on blood melatonin levels, with different research finding that it can result in an increase, decrease, or no change in the blood melatonin levels. It has also been found that, generally, age has an impact on one's blood melatonin levels. With a Repeated Measures experiment, I sought out to determine a conclusive answer on four questions:

- Does exercise impact blood melatonin levels?
- How does it impact blood melatonin levels?
- Does age truly impact blood melatonin levels?
- Do both variables have an interactive impact on blood melatonin levels?

Introduction:

Melatonin is a hormone produced by the body that manages the circadian rhythm and one's sleep schedule (National Center for Complementary and Integrative Health). Due to the importance of this hormone in everyday life, I was interested in researching what can potentially impact it within people.

While looking into the topic, I found that impacts of exercise on blood melatonin levels was "unclear" with research having "controversial" findings and that the results themselves vary wildly from study to study, with exercise having been found to cause an increase, decrease, and no change in blood melatonin levels across different experiments (Escames et al., 2011). With regards to the time of day, I also found that the impacts of exercise on melatonin levels could differ depending on what time of day it was completed at, with evening / night time exercise causing an increase in melatonin levels and afternoon exercise causing a non-significant shift in the levels (Buxton et al., 2003). Additionally, it was found that the intensity of the exercise could potentially cause a shift in results (Atkinson et al., 2003). In looking into other factors that could impact blood melatonin levels in combination with exercise, age was found to have a significant impact on the levels (Atkinson et al., 2003; Karasek et al., 2020).

With this research in mind, I decided to collect data in the afternoon only to have a consistent time of day to focus on and to investigate if afternoon exercise has no impact on melatonin levels. For the treatments, to account for the variance in impacts of exercises of different intensities, I decided to have the islanders either complete no exercise or to do a moderate activity (a thirty minute brisk walk). In considering the age variable, I decided to split the islanders included in the study into four age groups: < 18 (minors), 18-45 (adults), 45-65 (older adults), and > 65 (elders). This choice of exercise treatments and age groups signified that participants would be sorted into eight total groups for this experiment (with each group being a different exercise and age group combination).

Methods:

Participants

All samples collected are from islanders from the virtual “Islands” resource provided in our STATS 101B course. As I do not have a complete list of the islanders, I could not truly randomly sample the islanders such that every islander had an equal chance of being picked. With this in mind, for the purposes of this study, I attempted to randomly select islanders to the best of my abilities with the following three steps:

- Use the sample function in R to randomly select a city
- After selecting a city, use the same function to randomly select a house within that city
- Then, if that house had more than one person, randomly select a person within the household
    - If the person “randomly” selected did not consent to being a part of the study, I would repeat the selection process.

After an islander consented to being a part of the study, I flipped a coin to determine their treatment, with heads meaning that the islander would complete the brisk walk and tails meaning that the islander would complete no exercise (this, in turn, made this experiment a single-blind experiment). Using this entire process, I included 159 islanders in the experiment (20 in each exercise and age group combination except for the No Exercise & > 65 group in which only 19 samples were collected accidentally), which was derived from GPower and will be discussed further on.

Design

In formatting this Repeated Measures experiment, I decided to take blood melatonin measurements at three different points: before the treatment, five minutes after completing the treatment, and three hours after completing the treatment (this timeline is illustrated below). For the purposes of consistency between the exercise and no exercise groups, I considered the no exercise treatment length to also be thirty minutes (thus, the second measurement was taken thirty-five minutes after the first and the third was taken three-and-a-half hours after the first), The before measurement was taken as a baseline for each islander. I decided to collect data five minutes after treatment after noting that other studies used that as a post-exercise measurement point or simply took measurements immediately after completing treatment, such as Escames et al. and Diaz et al. in 1993 (Escames et al., 2011; qtd. in Buxton et al., 1997). The three hours after treatment measurement was also included after learning that “Montelone, et al. found that evening exercise led to significantly lower melatonin levels 3 hours after exercise” (qtd. in Pobocik et al., 2020).

Measurement One	After islander is selected and assigned treatment	
Complete Treatment	Exercise	No Exercise

Measurement Two	Five minutes after completion of treatment
Measurement Three	Three hours after completion of treatment

Instruments

Every step of the data collection process was completed through the virtual Islands previously mentioned (https://islands.smp.uq.edu.au/login.php). For the exercise treatment, the task used was "Brisk Walk Outdoors 30 mins", and for collecting the melatonin samples, I used the "Blood Melatonin" task.

Data Analysis:

Sample Size Determination

To determine how many islanders should be included in the experiment, I used G*Power and inputted the following parameters into it for the "MANOVA: Repeated Measures, within-between interactions" statistical test:

- Effect Size: 0.25
- α Error Probability: 0.05
- Power (1 - β Error Probability): 0.8
- Number of Groups: 8
- Number of Measurements: 3

With this information, GPower determined that I needed a minimum sample size of 153, which I then intended to round up to 160 to have an even 20 people per group.

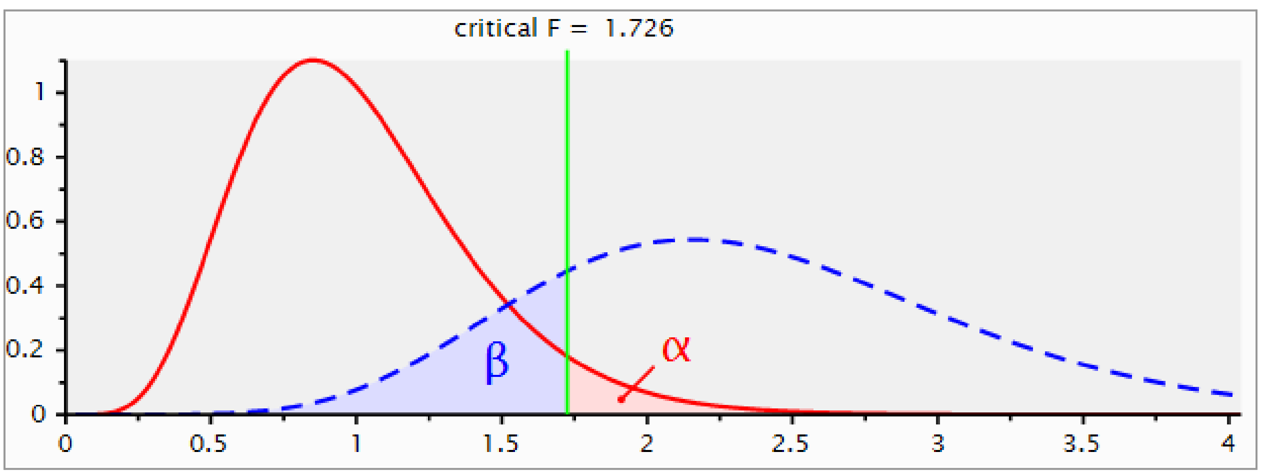

Figure 1: G*Power Output graph for our experiment.

Analysis Procedure

After all data was collected, I uploaded everything into RStudio. In RStudio, I created a model via the aov() function. From there, I analyzed the significance results of the model as well as the validity of our model.

Results & Analysis:

Data Graphs

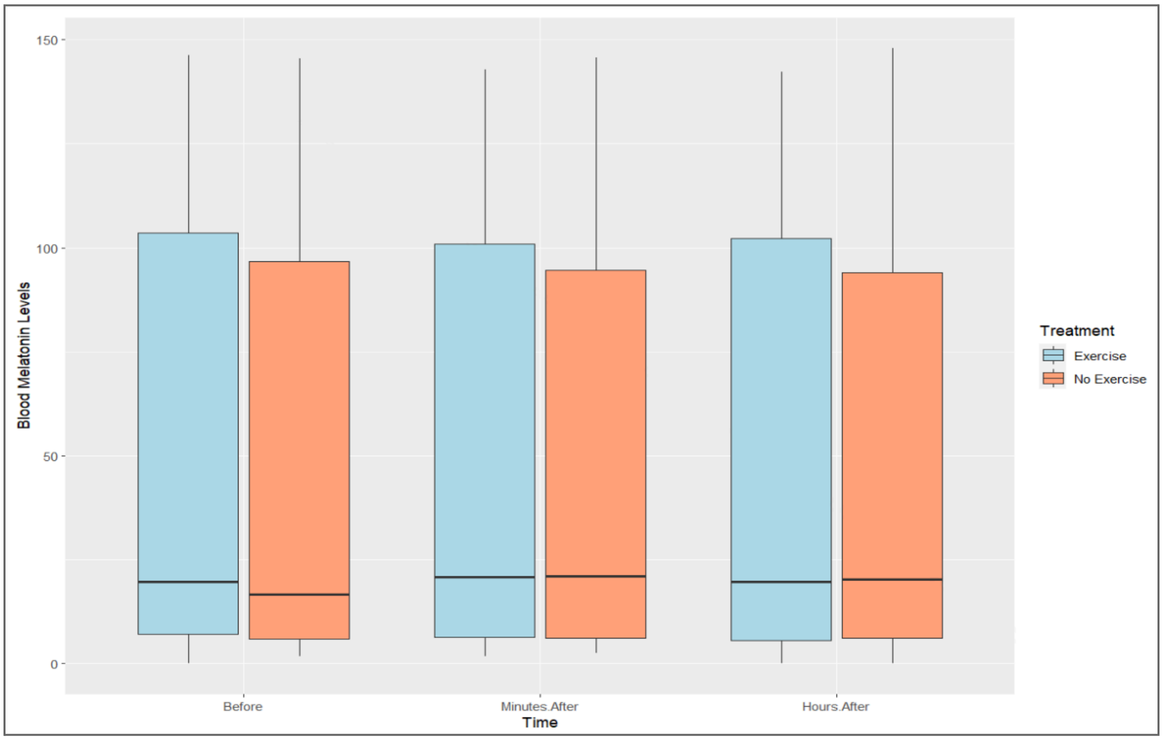

Figure 2: side-by-side boxplots of assigned treatment clustered by measurement time. The medians of each plot of data is noted by the black line through the middle of the “box”, the first and third quartiles by the lower and upper edges of the “boxes" respectively, and the minimums and maximum values by the lower and upper ends of the lines respectively

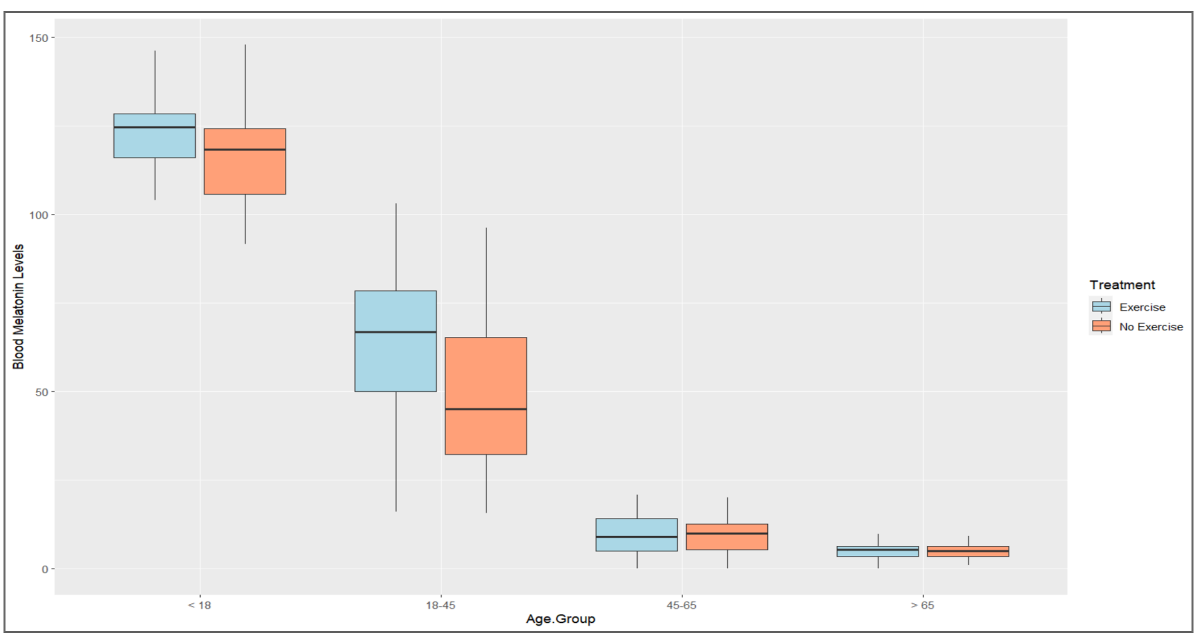

Figure 3: side-by-side boxplots of assigned treatment clustered by age group. The medians of each plot of data is noted by the black line through the middle of the "box", the first and third quartiles by the lower and upper edges of the "boxes" respectively, and the minimums and maximum values by the lower and upper ends of the lines respectively

With the first boxplot, there is a minimal difference in the treatment groups across the different times, with the most visible difference being that the no exercise groups generally have a lower interquartile range and third quartile than the exercise groups. Additionally, there is not a notable visual difference between the data across time in general. This initial illustrative analysis allows us to infer that there may be some significant difference in blood melatonin levels due to the exercise treatments and that there is likely not a significant difference within the data due to time.

With the second boxplot, we can easily infer that age group likely has a significant impact on the blood melatonin levels. Notably, there is no overlap between the < 18 and 45-65 boxplots, the < 18 and > 65 boxplots, and the 18-45 and the > 65 boxplots with there also being minimal overlap between adjacent age groups (besides 45-65 and > 65, which have a strong overlap). Additionally, there is a more clear visual difference in the exercise and no exercise data, with the no exercise groups generally having lower blood melatonin level values for the < 18 and 18-45 boxplots (as can be seen by the median of the exercise groups aligning with the third quartile of the no exercise groups). This new perspective of the potential impact of the treatments on blood melatonin levels allows us to infer that exercise may have a significant effect on melatonin levels.

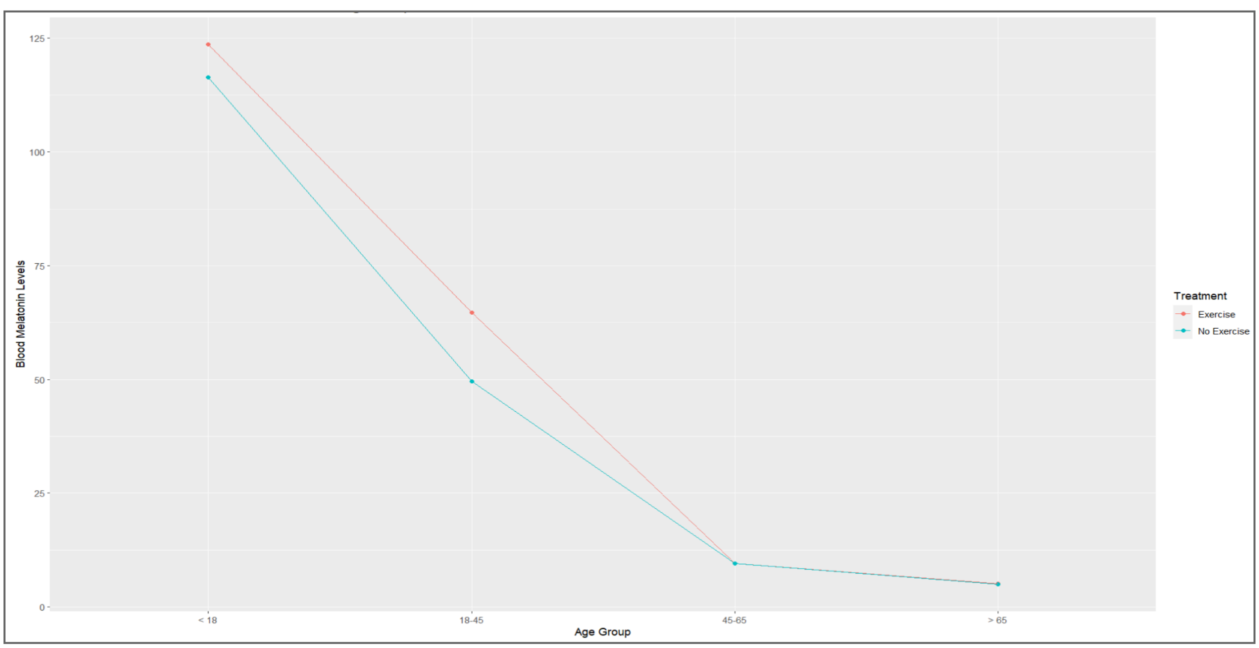

Figure 4: Interaction Plot of Blood Melatonin Levels with Treatment and Age Group.

There is an essentially perfect overlap between the no exercise and exercise lines from the 45-65 to the > 65 age groups, meaning that we can predict that there is likely a significant interaction effect from treatment and age group on the blood melatonin levels.

ANOVA Results

Error Between					
	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	P-Value
Treatment	1	3,075	3075	5.514	**0.0202**
Age Group	3	1,026,859	342,286	613.783	**< 2e-16**
Treatment & Age Group	3	4,587	1,529	2.742	**0.0453**
Residuals	151	84,208	558		
Adjusted R^2: 0.924					

Table 1: Error Between Groups ANOVA Table. Significant P-Values are bolded.

Error Within					
	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	P-Value
Time	2	0.1	0.036	0.009	0.9909
Time & Treatment	2	2.6	1.292	0.331	0.7187
Time & Age Group	6	49.1	8.182	2.093	0.0539
Time & Treatment & Age Group	6	19.9	3.312	0.847	0.5342
Residuals	302	1,180.3	3.908		

Table 2: Error Within Groups ANOVA Table. Significant P-Values are bolded.

As predicted when looking at the box plots and interaction plot for the data, treatment, age group, and the interaction between the two are all significant with p-values of 0.0202, essentially 0, and 0.0453 respectively. The model also has an adjusted R^2 value of 0.924, meaning that 92.4% of the variance in the blood melatonin levels can be explained by the model.

Also as predicted from the boxplots, time does not have a significant effect on the blood melatonin levels with it having an extremely high p-value of 0.9909, and none of the interactions between time and the independent variables in the experiment are significant.

Tukey HSD Adjusted P-Values

The Exercise group has a mean difference from the no exercise group of 7.51, meaning that the no exercise group generally had lower blood melatonin levels than the exercise group.

Age Groups

Age Group Pair	P-Value
< 18 & 18-45	**0.00**
< 18 & 45-65	**0.00**
< 18 & > 65	**0.00**
18-45 & 45-65	**0.00**
18-45 & > 65	**0.00**
45-65 & > 65	**0.0449**

Table 3: Tukey HSD P-values for differences between age groups. Significant P-Values are bolded.

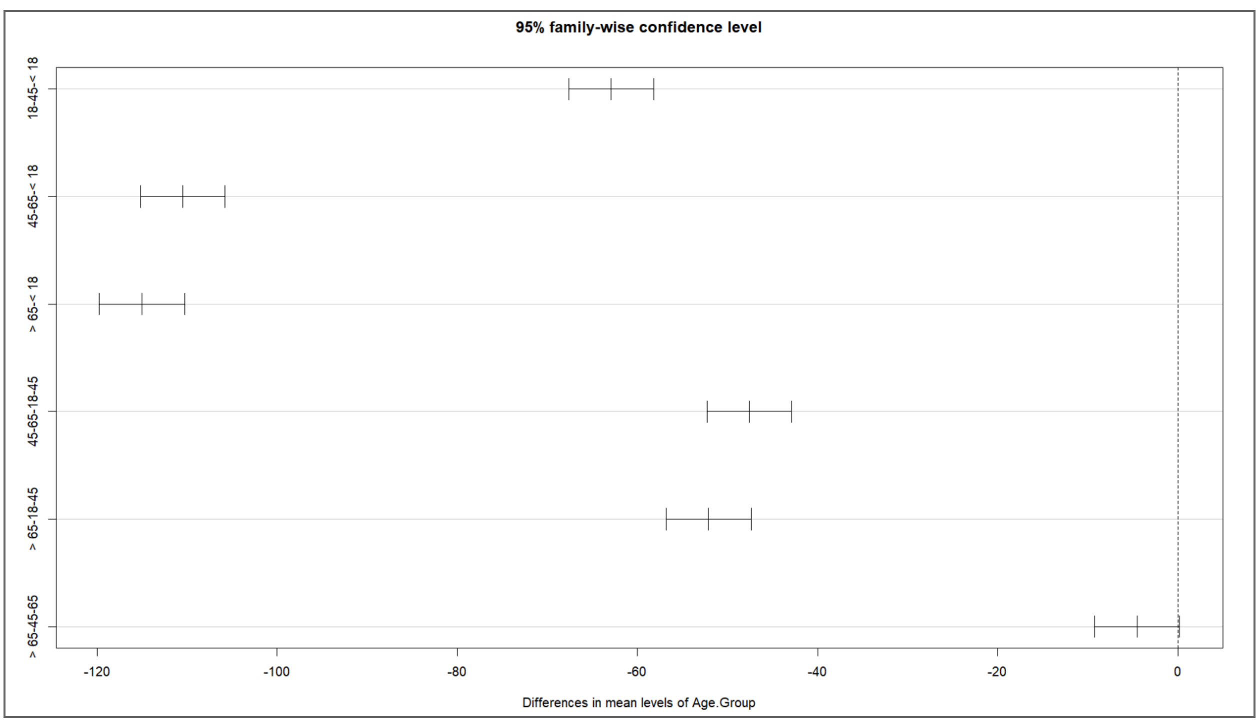

Figure 5: Tukey HSD Plot for Age Groups.

The first post-hoc table shows that every age group combination is significantly different. Additionally, every combination (except for the 45-65 and > 65 age group combination) has a p-value of 0.00. From our graph of the Tukey HSD differences between groups, we can see that the strongest differences come from the < 18 and 45-65 age group combination and the < 18 and > 65 age group combinations, with them both having a mean difference of over 100 pg/ml. Also from the graph, we can see that the 45-65 and < 65 age group combination mean level differences does have a slight overlap with 0, which explains the combination's lower p-value in comparison to the rest of the combinations. Additionally, the graph shows that as the age groups increase, the blood melatonin levels decrease.

Treatment & Age Group Interaction

Treatment & Age Group Combination	P-Value
E < 18 & NE < 18	0.074
E < 18 & E 18-45	**0.00**
E < 18 & NE 18-45	**0.00**
E < 18 & E 45-65	**0.00**
E < 18 & NE 45-65	**0.00**
E < 18 & E > 65	**0.00**
E < 18 & NE > 65	**0.00**
NE < 18 & E 18-45	**0.00**
NE < 18 & NE 18-45	**0.00**
NE < 18 & E 45-65	**0.00**
NE < 18 & NE 45-65	**0.00**
NE < 18 & E > 65	**0.00**
NE < 18 & NE > 65	**0.00**
E 18-45 & NE 18-45	**0.00**
E 18-45 & E 45-65	**0.00**
E 18-45 & NE 45-65	**0.00**
E 18-45 & E > 65	**0.00**
E 18-45 & NE > 65	**0.00**
NE 18-45 & E 45-65	**0.00**
NE 18-45 & NE 45-65	**0.00**
NE 18-45 & E > 65	**0.00**
NE 18-45 & NE > 65	**0.00**
E 45-65 & NE 45-65	1.00
E 45-65 & E > 65	0.595
E 45-65 & NE > 65	0.596
NE 45-65 & E > 65	0.611
NE 45-65 & NE > 65	0.611
E > 65 & NE > 65	1.00

Table 4: Tukey HSD P-values for differences in treatment and age group combinations. Significant P-Values are bolded. Label key: the exercise treatment is labeled as E and no exercise is labeled as NE

Our second post-hoc table for the interaction between treatment and age group shows that a strong majority of our treatment and age group combinations are significantly different from the other combinations, with exceptions arising in all of the 45-65 and > 65 age group combinations and the two treatments for the < 18 age groups. Every significant pairing also has a p-value of 0.

Diagnostic Plots

Original Model

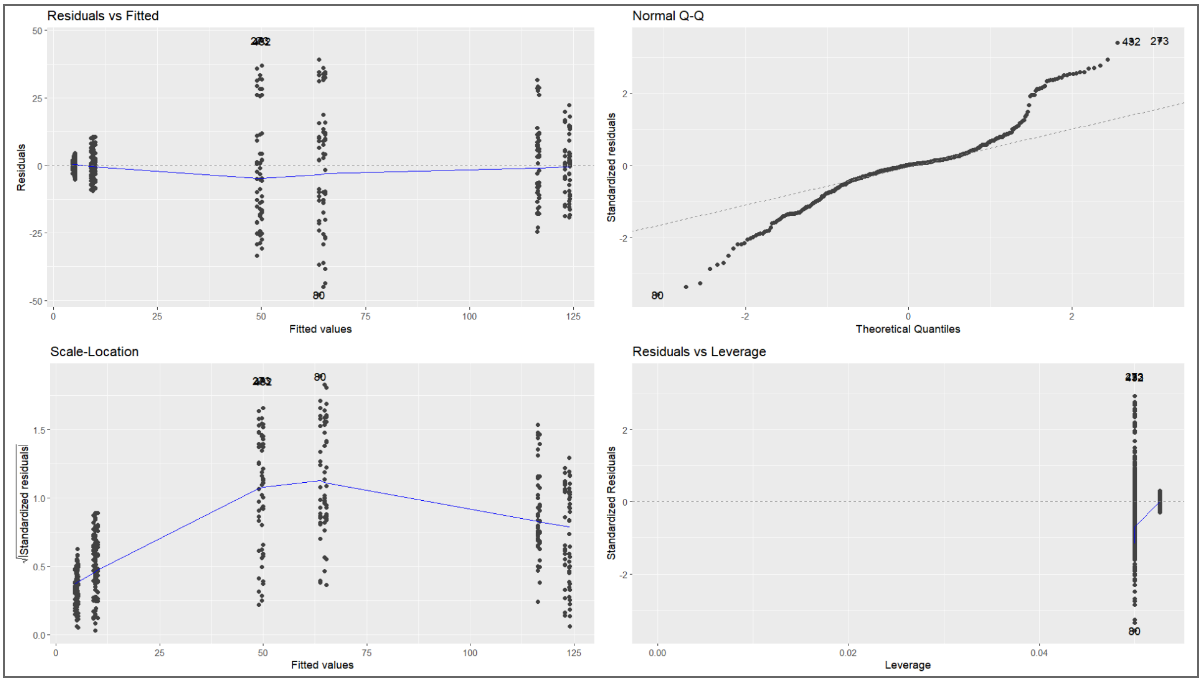

Figure 6: Diagnostic plots for our model.

Box-Cox Transformation

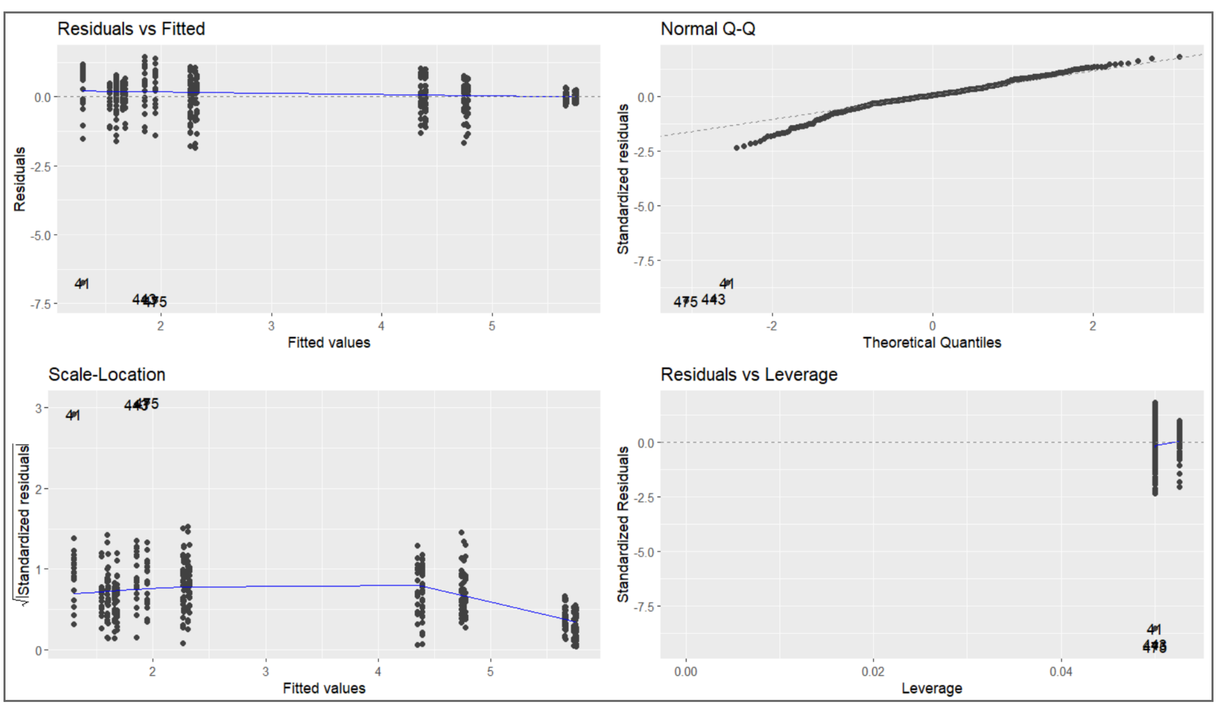

Figure 7: Diagnostic plots for our model after completing a box-cox transformation ($\lambda = 0.0719$).

In our diagnostic plots for our original model, the assumption of linearity, but the assumptions of normality and constant variance both are likely violated as the points deviate on both ends of the Normal Q-Q line and the best-fit line in the Scale-Location plot shifts across the fitted values. To correct this, I did a Box-Cox transformation. I found that the best lambda for the model was 0.0719, adjusted the blood melatonin values accordingly, created a new model with this transformed data, and created new diagnostic plots.

In the new diagnostic plots, all assumptions are more adequately fulfilled. Points follow the Normal Q-Q line more consistently, with the exception of a few outliers below the -2 quantiles. The best-fit line in the Scale-Location plot is more consistent as well, with it only slightly shifting towards the higher fitted values. The Residuals vs Leverage plot confirms the existence of a few outliers within the data as some points lie outside the -2 to 2 standardized residual rate, with the points that deviate in both the Normal Q-Q and Scale-Location plots having standardized residuals of around -8.

Conclusions:

Throughout this experiment and paper, I successfully answered all four of my initial research questions:

- Does exercise impact blood melatonin levels?
    - Yes, daytime exercise does impact blood melatonin levels.
- How does it impact blood melatonin levels?
    - Daytime exercise results in an increase in blood melatonin levels.
- Does age truly impact blood melatonin levels?
    - Yes, age impacts blood melatonin levels. Generally, as the age groups increase, the blood melatonin levels decrease.
- Do both variables have an interactive impact on blood melatonin levels?
    - Yes, there is a strong interaction effect of both variables on blood melatonin levels.

There are improvements to be made in this experiment as well as directions in which this can be taken in the future.

For the former, there are two immediate limitations that I noted throughout the experiment and in analyzing the data. First, the data was collected throughout the afternoon, but I had no set start and stop times for collecting the first set of data and starting treatments due to being a student with a schedule that varies day-by-day; ideally, in the future, this can be adjusted to make the data collection more consistent. Second, I made an error in counting how much data I collected; while I meant to collect data from 160 islanders, I only collected data from 159 islanders and did not notice until it was too late to collect the missing data. Thankfully, I had enough data still to complete my analysis with an adequate sample size, but in the future, hopefully this issue does not recur.

For the latter, there are two notable ways I can envision this experiment being developed in the future: by completing the same experiment over a much longer period of time and by completing the experiment with different potential independent variables. In doing this experiment again across a longer time period (such as a month or longer), we could see if daily exercise has a lasting effect on blood melatonin levels and if stronger differences arise between the no exercise and exercise groups. By experimenting with different factors, we can learn more about what impacts blood melatonin levels in combination with exercise (or simply on its own).

Works Cited

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