Effects of Dark Chocolate Cocoa Percentage and Digestion Interval on Short-Term Memory Performance in Male Islanders

Group C
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1 Abstract

Previous studies have found benefits in cognitive function from consuming chocolate. This study investigates whether varying cocoa percentages in chocolate enhance male memory performance, using a randomized complete-block design (5 cocoa levels × 3 digestion intervals x 5 age blocks) in a sample drawn from the town of Colmar ($n = 150$). Memory performance was assessed through a Memory Game which was completed before and after the assigned chocolate consumption and digestion duration. We found no significant difference in Memory game completion time despite consuming different levels of chocolate. However, we found that the digestion interval (the time between eating the chocolate and taking the second Memory Game test) was significant ($p < 0.05$), with shorter intervals associated with faster completion times. Although age was not the focus of our study, we also found it to be a significant blocking factor that explained a significant amount of variation in memory performance ($p < 0.05$).

2 Introduction

Chocolate has been consumed for thousands of years and is valued not only for its sweet taste, but also for its medicinal and functional properties. In recent years, chocolate has been researched for its benefits to the cardiovascular system and cognitive abilities (Crichton et al.). Indeed, a systematic review by Shateri et al. found that regular consumption of chocolate improves executive function in adults. Similarly, other studies have shown that dark chocolate improves verbal working memory for students in the short-term (Prastowo et al.).

These cognitive benefits are largely attributed to cocoa flavanols, a type of flavonoid, which is a naturally occurring plant-based compound present in chocolate. Cocoa flavanols fall in the polyphenol family, which increase cerebral blood flow and are associated with improved cognitive function (Sasaki et al.). Flavanol concentrations vary with cocoa content, making cocoa percentage a relevant factor in assessing chocolate's cognitive effects (Socci et al.).

Furthermore, we were also interested in determining if the length time after consuming chocolate would have a significant effect on the ability to play the memory game faster. Studies have shown that blood flow to the brain increases after ingesting flavanols, with a peak approximately two hours after a consumption (Francis et al.). Therefore, we decided to assign participants to play the memory game again at one of three times: right after chocolate consumption, one hour after consumption, or two hours after consumption.

As students, we were especially interested in seeing if chocolate could be used as a tool to improve memory as this would greatly support academic performance. Despite growing research on these effects, gaps remain in the literature. Research has disproportionately focused on females, such as a study by Lamport et al. Furthermore, few studies have examined how age-related differences in cognition may influence chocolate's impacts. To explore this question, we designed this study to focus on short-term memory outcomes in male participants. We also utilized age as a blocking factor, due to the known changes in cognitive and memory ability during aging. By addressing these gaps, our study aims to contribute to the literature by focusing on short-term memory across age groups.

With regards to our first treatment factor, we hypothesize that higher cocoa levels will produce a greater improvement in memory game performance. With regards to our second treatment factor, we also hypothesize that the largest difference in time to play the memory game will be at two hours after consumption of chocolate.

3 Methods

3.1 Participants

Participants in our study were drawn from the virtual environment known as *The Islands*. Specifically, we sampled male participants from five age brackets residing in the village of Colmar on Bonne Santé Island. After collecting our sample, each participant was randomly assigned to one of the 15 combinations of treatments using R.

3.2 Design

The study was designed as a Randomized Complete Block Design with two factors and one block, resulting in 75 groups.

Response Variable	Difference in Memory Game performance				
Treatment 1 (Chocolate Level)	White	40% cocoa	70% cocoa	85% cocoa	99% cocoa
Treatment 2 (Time Interval)	Right after	One hour	Two hours		
Block (Age Bracket)	10-19	20-29	30-39	40-49	50+

Figure 1: Parameters of the study's design.

The response variable is the difference in time between performance on the Memory Game before and after chocolate administration. The chocolate level is the type of chocolate that the Islander is to consume. The time interval denotes how long the Islander is to wait after eating the chocolate before completing the second Memory Game.

	Right After	One Hour	Two Hours	Total
White	10 Participants	10 Participants	10 Participants	30 Participants

40% Cocoa	10 Participants	10 Participants	10 Participants	30 Participants
70% Cocoa	10 Participants	10 Participants	10 Participants	30 Participants
85% Cocoa	10 Participants	10 Participants	10 Participants	30 Participants
99% Cocoa	10 Participants	10 Participants	10 Participants	30 Participants
Total	50 Participants	50 Participants	50 Participants	150 Participants

Figure 2: Factor design for each level of Chocolate and Time Interval. Each of the 15 chocolate × digestion combinations has two people from each age bracket (10-19, 20-29, 30-39, 40-49, 50+), making 10 total participants per cell.

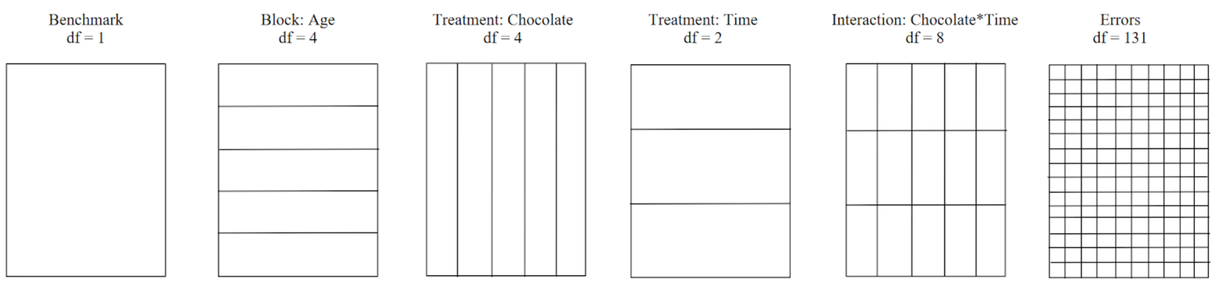

Figure 3: Factor diagram.

3.3 Instruments

Cognitive and memory performance were assessed using a standardized Memory Game, which required participants to play a paired memory game to correctly match 15 pairs (30 cards) as fast as they could. Completion time, measured in seconds, was automatically recorded and presented by *The Islands*'s interface.

Chocolate samples were administered in standardized 50g portions across five cocoa concentration levels: White chocolate, 40%, 70%, 85%, and 99% dark chocolate. The participants then fully consume the chocolate, usually within 1-2 minutes.

To evaluate the effect of time after consumption on memory performance, participants completed the second memory game at one of three designated intervals: immediately after, one hour after, or two hours after chocolate intake. For participants that were assigned to complete the second memory game one hour after or two hours after consumption, researchers began a timer immediately after chocolate consumption to ensure the second memory game was conducted on time.

3.4 Procedure

Step 1: Find male subjects from Colmar within the age brackets specified and ask them for consent to participate in the experiment.*

Step 2: In each age bracket, use R to randomly assign each subject to, first, a chocolate level and then a time interval.

Step 3: Have each subject complete the Memory Game and record their time.

Step 4: Then, apply the assigned treatment to the islanders by having them consume their assigned chocolate level.

Step 5: After the islander has finished consuming the chocolate, have the subject wait according to their assigned time interval before they complete the Memory Game one more time.

Step 6: For each subject, compute the difference in time to complete the Memory Game by subtracting the time taken pre-treatment from the post-treatment results. **

** All data was collected from between 4 p.m. and 8 p.m. so that time of day does not impact the results of the study.*
*** A more negative difference indicates that participants improved on the Memory Game, that is, participants matched the cards more quickly on the post-treatment test.*

4 Data Analysis

4.1 Type of Statistical Analysis

To conduct our exploratory data analysis and create appropriate data visualizations, we used R, certain packages in RStudio (dplyr, ggplot2, ggfortify), and LaTeX.

Utilizing the F-tests in ANOVA, we will test if there is a significant difference between the time to complete the Memory Game before and after the chocolate consumption and assigned consumption waiting time. Moreover, we will also examine if there is an interaction between chocolate level and time interval, and whether or not age as a blocking factor is significant. The following multiple linear regression model specification was used for analysis:

$$Y = \beta 0 + \beta 1(\text{Chocolate}) + \beta 2(\text{Time}) + \beta 3(\text{Chocolate}\times\text{Time}) + \text{Block}(\text{Age}) + \varepsilon$$

4.2 Sample Size Determination

We utilized the power of 0.8, which is the probability of correctly rejecting the null hypothesis when it is false. Moreover, we used the alpha significance level of 0.05. The effect size of 0.4 was determined by following the effect size used by previous studies (Banaei et al.). Using G*Power, we determined that the minimum sample size required is n = 114 based on the degrees of freedom of the interaction (4 x 2 = 8). However, since we have 75 different combinations between chocolate level, time interval, and age block (5 x 3 x 5 = 75), our sample size will be n = 150, where each unique combination will have 2 subjects.

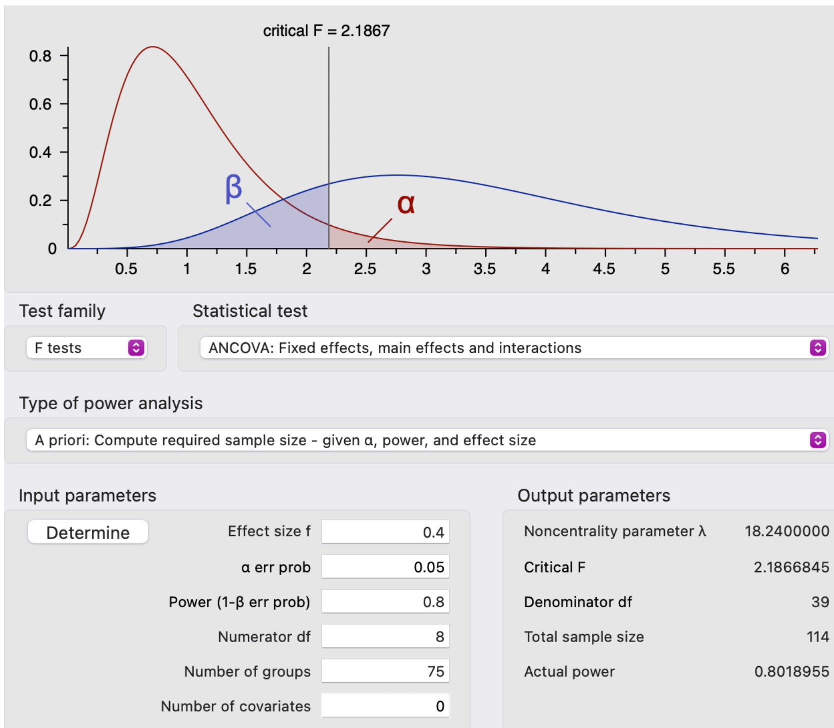

Figure 4: G*Power output for sample size determination with d= 0.4, power = 0.8 and α = 0.05, df = 8.

5 Results

5.1 Data Description

Chocolate Level	Mean Difference	Standard Deviation	n
White	-2.40	6.64	30
40% Cocoa	-3.33	7.64	30
70% Cocoa	-3.54	8.52	30
85% Cocoa	-2.89	6.87	30
99% Cocoa	-2.15	6.65	30

Figure 5: Descriptive Statistics for Chocolate Levels.

Mean Difference is the mean of the difference between the time taken for the second memory game and the time taken for the first game for each chocolate level. We can see negative values quite close to each other between -3.537 and -2.153.

Time Interval	Mean Difference	Standard Deviation	n
Right After	-4.69	9.29	50
One Hour	-2.82	5.05	50
Two Hours	-1.08	6.33	50

Figure 6: Descriptive Statistics for Time Intervals.

Mean Difference is the mean of the difference between the time taken for the second memory game and the time taken for the first game for each time interval which ranges between -1.08 and -4.69.

5.2 Box Plots

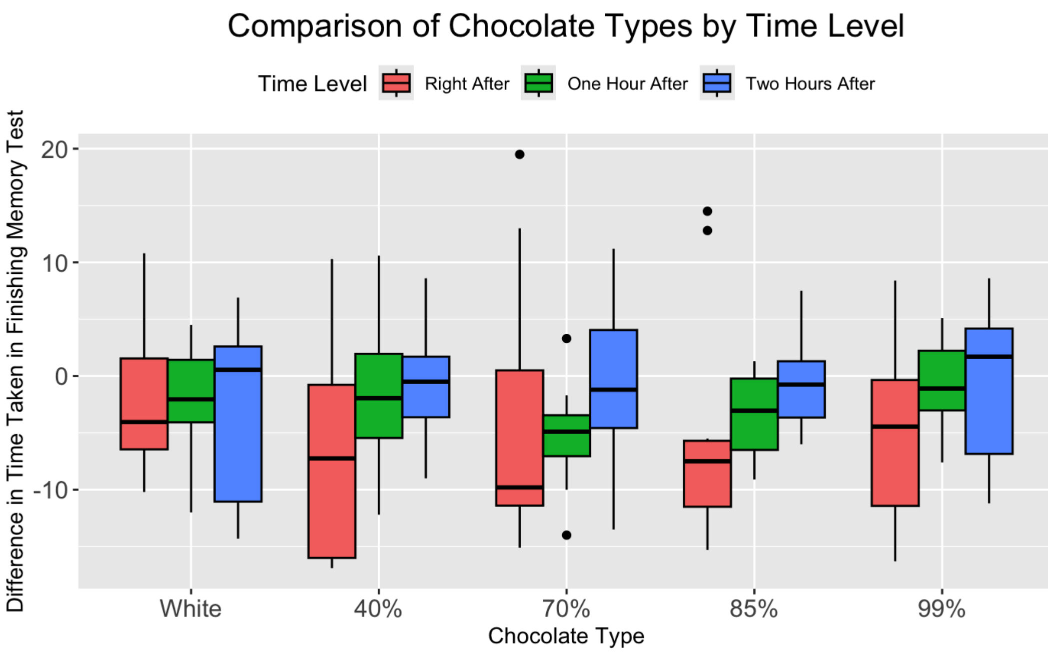

Figure 7: Comparing Chocolate Types (White, 40%, 70%, 85%, 99%) by Time Interval (right after, one hour after, two hours after). The black dots represent outliers.

We displayed the box-plots distribution for each chocolate level and within each, the three different time intervals. Figure 7 shows overlapping boxplots with some differences in the medians between chocolate types. In general, the greatest difference (most negative values) occurs in the medians recorded immediately after eating chocolate.

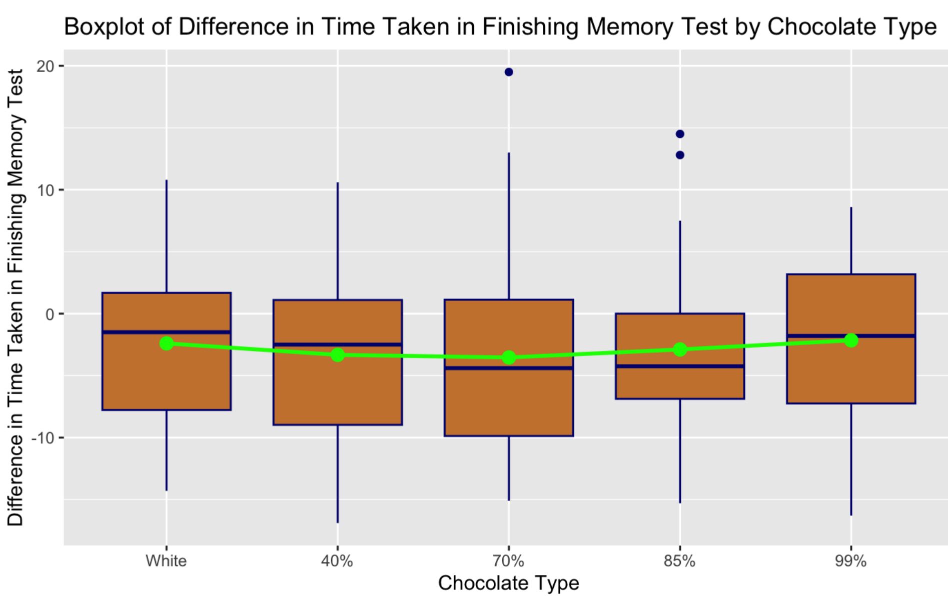

Figure 8: Boxplot of Difference in Time Taken in Memory Game for each Chocolate Type with the green line showing the mean for each Chocolate Type. The black dots represent outliers.

Collectively, the median and mean difference in time taken in doing the memory test for each Chocolate Type is close to each other thus, suggesting a low significance in chocolate cocoa level affecting cognitive abilities.

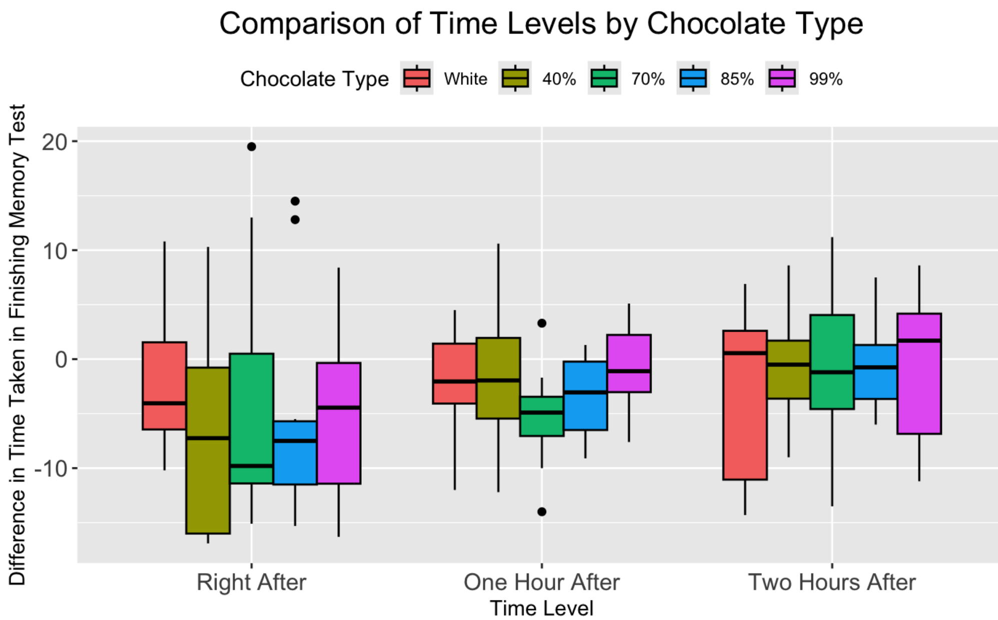

Figure 9: Comparing Time Intervals (right after, one hour after, two hours after) by Chocolate Type (White, 40%, 70%, 85%, 99%). The black dots represent outliers.

We also split the medians up by each time interval, and displayed the distribution of the 5 different chocolate types eaten by the islanders. However, the median difference of each chocolate type is relatively close to each other.

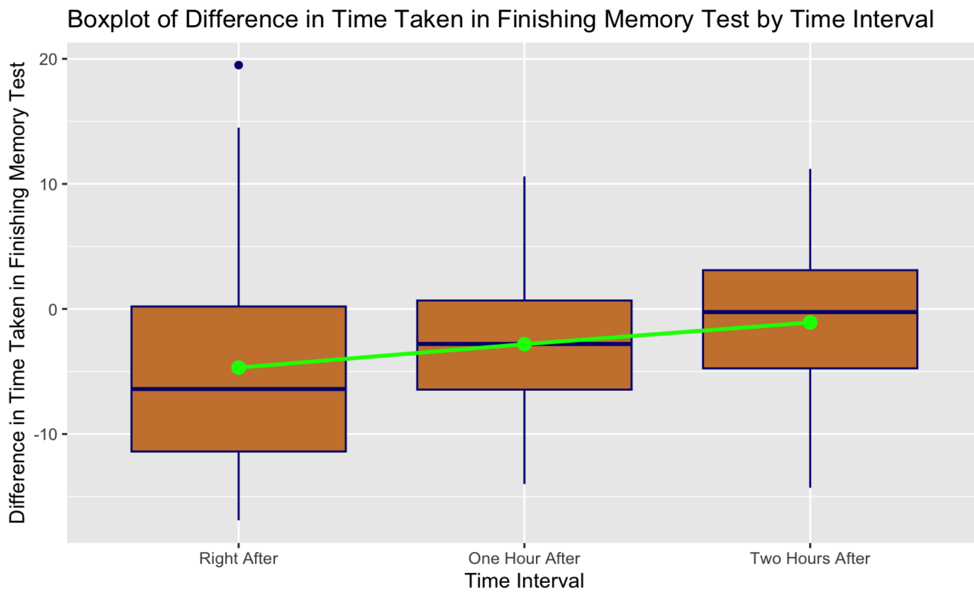

Figure 10: Boxplot of Difference in Time Taken in Memory Game for each Time Interval with the green line showing the mean for each Time Interval. The black dot represents an outlier.

Collectively, the median difference in time taken in doing the memory test for each Time Intervals increases with time, given by both the median and mean. This indicates that the Right After group had the most negative (highest differences) while the Two Hours After group had the least difference between the time it took to complete the first memory game and the time it took to complete the second memory game.

5.3 Interaction Plot

Figure 11: Interaction Plot of Chocolate Type and Time Interval with Difference in doing the memory after each Time Interval.

Each line in the plot represents a Chocolate Type. This graph suggests that there may be interaction between the Chocolate Types as the lines are not parallel to each other.

5.4 ANOVA Analysis

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Chocolate Level	4.0000	41.6257	10.4064	0.2495	0.9095
Time Level	2.0000	326.3177	163.1589	3.9118	**0.0224**
Age Range	4.0000	1692.6137	423.1534	10.1452	**3.47e-07**
Chocolate Level and Time Level	8.0000	243.4743	30.4343	0.7297	0.6651
Residuals	131.0000	5463.9643	41.7097		

Figure 12: ANOVA Results

Based on the ANOVA analysis, chocolate level (p-value: 0.9095) was not a significant treatment factor at a significance level of 0.05. Therefore, we fail to reject the null hypothesis that islanders that consume all levels of chocolate have statistically equal mean differences in the time it takes to finish the memory test.

However, time interval was statistically significant as the p value of 0.0224 is below the threshold of 0.05. In this case, we reject the null hypothesis that all time intervals have a statistically equal average effect on the difference of time it takes to complete the memory test.

There is statistically significant evidence to suggest that at least two of the group's average differences are statistically different.

Finally, as a blocking factor, age was very significant, as its p-value of 3.47e-07 is much below the significance level of 0.05. Since blocking is used to control a nuisance variable that may affect the response variable, our group was able to significantly reduce residual standard error through an effective blocking factor.

As interaction is not tested with blocking factors, only the interaction between our two treatment variables, chocolate level and time interval, was included in our ANOVA analysis. The interaction term was also not statistically significant, as the p-value 0.6651 is above the significance level of 0.05. This indicates that chocolate level and time interval do not depend on each other when affecting the difference in time taken to complete the memory test. Unlike the interaction plot where the non-parallel lines may have hinted at the possibility of interaction between chocolate level and time interval, the ANOVA table demonstrates that this level of interaction is actually not significant and the effect of each treatment factor on the response variable can be interpreted independently.

5.5 Tukey HSD Adjusted P-values

Based on the ANOVA results, we determined that time interval was a significant factor that had an effect on the mean difference in time it took to complete the memory test. In order to determine which exactly of the three groups of time intervals was different from the other group means, we conducted a Post-Hoc Tukey HSD test for pairwise comparisons.

Comparison	Difference	Lower	Upper	P Value Adjusted
Right After and One Hour After	-1.874	-4.936077	1.188077	0.3180854
Two Hours After and One Hour After	1.738	-1.324077	4.800077	0.3726983
Two Hours After and Right After	3.612	0.5499234	6.674077	**0.0162804**

Figure 13: Tukey HSD test for pairwise comparison between the three levels of time interval.

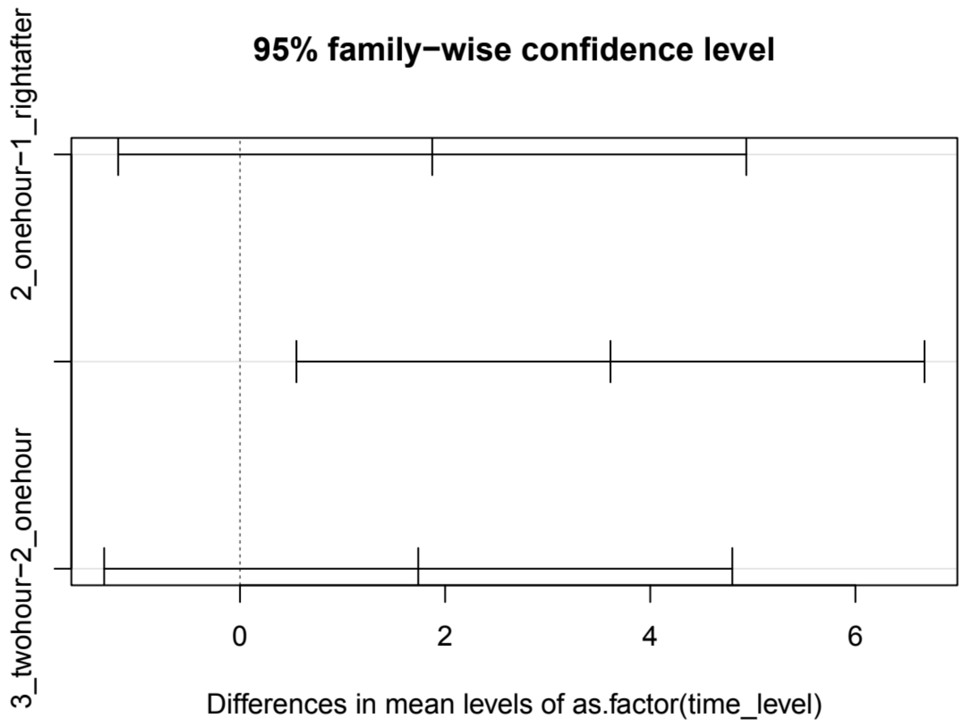

Figure 14: Confidence Interval for Pairwise Comparisons of Tukey HSD results for Time interval.

Based on the Tukey HSD results, only the significant difference is between right after and two hours. The p-value for this pairwise comparison is 0.01628, which means we reject the null hypothesis that the two group means are equal. This is further corroborated by Figure 14, the graph of the confidence interval, where the confidence interval for right after and two hours after does not cross 0. However, the confidence interval for one hour after and two hours after, as well as the confidence interval for right after and one hour after both cross 0, which means the group means are not statistically different. This is consistent with the p-values, which one hour after and two hours after is 0.37269, and for right after and one hour after is 0.31808. Both of these are above the significance level of 0.05, so we fail to reject the null hypothesis that the difference in those group means are statistically equal.

Next, we decided to check which levels of our age group block significantly differed from others as we concluded that age was a significant blocking factor from the ANOVA results.

Comparison	Difference	Lower	Upper	P Value Adjusted
20 to 29 year olds and 10 to 19 year olds	7.9333333	3.320721	12.545946	**0.0000496**
30 to 39 year olds and 10 to 19 year olds	0.1966667	-4.415946	4.809279	0.9999558
40 to 49 year olds and 10 to 19 year olds	-1.3366667	-5.949279	3.275946	0.9297008
50+ year olds and 10 to 19 year olds	-0.3000000	-4.912612	4.312612	0.9997627
30 to 39 year olds and 20 to 29 year olds	-7.7366667	-12.349279	-3.124054	**0.0000810**
40 to 49 year olds and 20 to 29 year olds	-9.2700000	-13.882612	-4.657388	**0.0000014**
50+ year olds and 20 to 29 year olds	-8.2333333	-12.845946	-3.620721	**0.0000231**
40 to 49 year olds and 30 to 39 year olds	-1.5333333	-6.145946	3.079279	0.8889256
50+ year olds and 30 to 39 year olds	-0.4966667	-5.109279	4.115946	0.9982651
50+ year olds and 40 to 49 year olds	1.0366667	-3.575946	5.649279	0.9713838

Figure 15: Tukey HSD test for pairwise comparison between the five levels of age blocks.

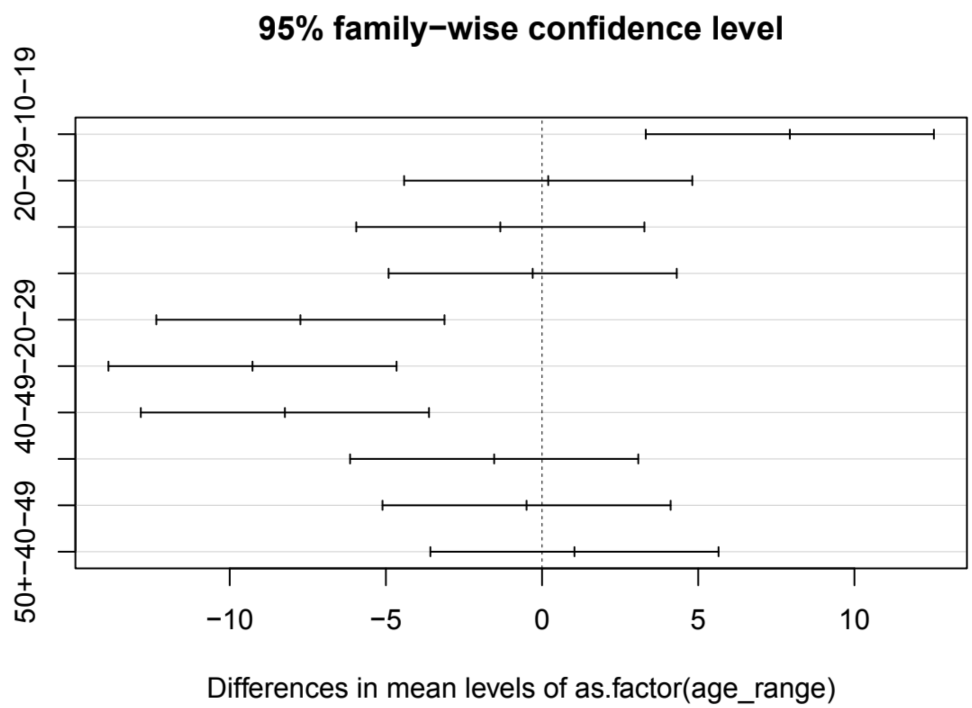

Figure 16: Confidence Interval for Pairwise Comparisons of Tukey HSD results for Age Block.

From Figure 15, we can see that only the 20-29 year old age group differed significantly from the average means of all other age groups. The p-value for the pairwise comparison with 10-19 year olds was 0.0000496, with 30-39 year olds was 0.0000810, with 40-49 year olds was 0.0000014, and with 50+ year olds was 0.0000231. All of these p-values are much below the significance level of 0.05, which means that we reject the null hypothesis that the two group means in each pairwise comparison are statistically equal. In Figure 16, we can see that these groups do not cross 0, further indicating that they have a significant difference. All of the other age group pairwise comparisons were not significantly different as their p-value was above the significance level. Additionally, all of these groups' confidence intervals crossed 0, indicating that they are not significantly different.

5.6 Residual Diagnostics

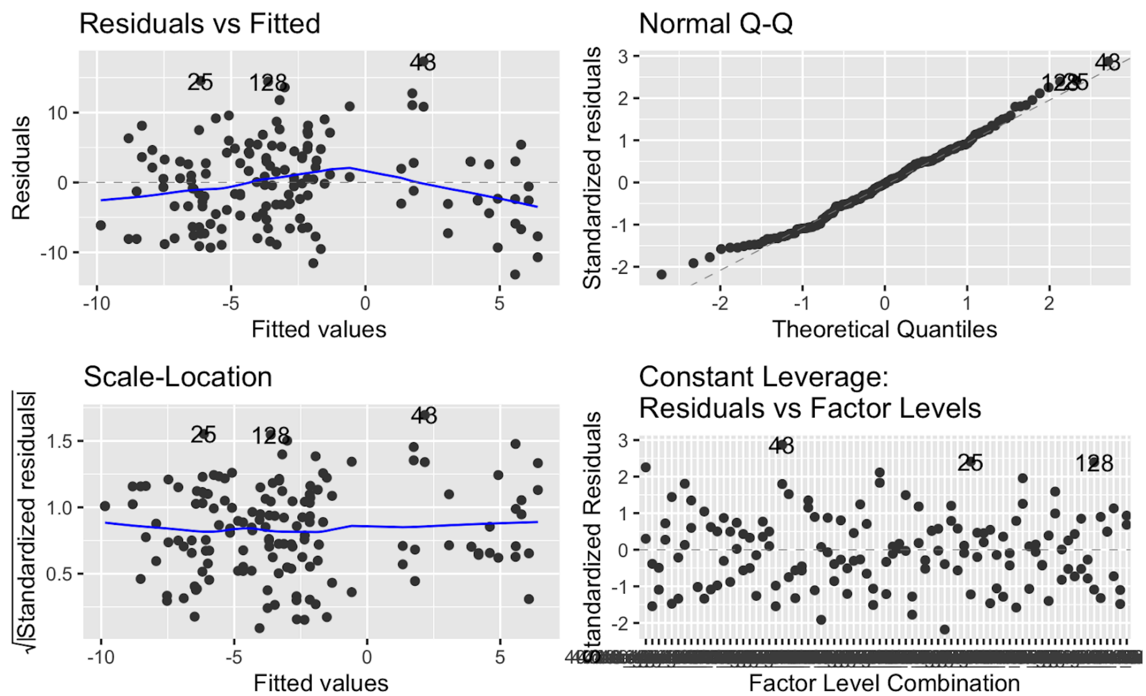

Figure 17: Residual Summary Plots for the Model. Each plot from top left to bottom right shows plots for the linearity, normality, constant variance assumptions, while the last plot displays the presence of outliers.

The first plot shows that the blue line is nearly horizontal, satisfying the linearity assumption. The QQ-Plot has most on the line except for a few at the bottom left extreme and top right extreme. This still satisfies the normality assumption. Also, the Scale-Location plot is nearly flat and thus, the model has constant variance. There are only 3 outliers noted by R on the last plot.

6 Discussion

The aim of this study was to determine whether different cocoa percentages in dark chocolate and the time waited after consuming chocolate impacts performance on a memory game involving matching 30 cards. Previous studies have shown that dark chocolate can improve cognitive functions, including memory and attention. Our results attempt to identify which type of dark chocolate and consumption time is most effective at improving memory.

Based on our G*Power calculation, we needed at least 114 participants to achieve an 80% power with an assumed effect size of 0.40 derived from Banaei et al. We instead sampled 150 participants for a balanced design, which yielded around a 94% power under the same effect-size assumption. If the true effect size were closer to 0.33, 150 participants would still give over an 80% power. However, since we ultimately used an effect size of 0.4, oversampling by 34 reduces the risk of being underpowered if the actual effect size fell below 0.40.

Male participants were sampled from Colmar by age group to ensure no individuals were sampled twice, and each individual was randomly assigned to a treatment group. After conducting the experiment, we imported data into R to conduct data analysis. Our initial exploration of the data through boxplots revealed that chocolate level seemed to have no effect on memory game performance, while time interval did.

We followed up this exploration with a linear model with both factors and age as a block, which confirmed our observations of the boxplot. Time interval has a p-value of 0.0224, and age block has a p-value of >0.001, indicating that both are significant. Chocolate type was not significant. To analyze our model more in depth, we conducted a post-hoc Tukey HSD test on time interval and age block since those were the only significant factors. The Tukey test revealed that taking the test right after consuming chocolate had significant differences from taking it after two hours. Additionally, for the age block, the 20-29 age group was significantly different from all other age groups. Finally, analysis of diagnostic plots indicated that all model assumptions were met, making these results valid. These results indicate that cocoa level has no impact on memory game performance, while time waited between memory games and age does impact performance. Therefore, we fail to reject the null hypothesis that chocolate percentage has no effect on short term memory, while we reject the null hypothesis that digestion time has no effect on short term memory.

We would like to recognize some broader limitations in our experiment, namely the virtual environment, memory game, and design type. This experiment was conducted in a virtual environment, *the Island*, which does not perfectly imitate real life. As researchers, we have relatively limited control over how the experiment is administered, for example, the setting where chocolate is given, participant preference for chocolate, etcetera. Additionally, the Island may not be programmed to respond to chocolate, potentially reflecting the lack of significance of dark chocolate on *the Island* while real-life studies reveal its positive effects on memory. Next, the memory game involves visual and spatial memory which has not been analyzed as rigorously in relation to chocolate consumption. *The Island* offers limited choices for memory related tests, and this one may not have been the best reflection of practical memory benefits in real life. We also are unsure whether the same cards are used in every test, or if variations of the test are administered. Finally, our design was intended to explore the interaction between the two factors to gain an understanding of whether chocolate type and digestion time impacts memory performance. However, we did not consider person to person variability which is an important consideration for memory related experiments. A Latin Squares or Repeated Measures design may be appropriate for future research, especially studies investigating cocoa level specifically.

For future experiments, we recommend using an alternative design method that focuses more on person to person variability. In addition, using a control group who consumed no chocolate could be helpful in investigating whether any level of chocolate impacts memory on *the Island*. In our experiment, we also found that Islanders performed best the sooner they took the second memory test. This may indicate that chocolate has no impact, but performance is improved on memory matching games with practice. Continuing to research the impact of repetition on memory game performance and the impact of chocolate on other cognitive processes are interesting directions for future research.

7 References

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