Impacts of Dark Chocolate with Varied Cocoa Content and Dosage on Urine Dopamine Levels

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1 Abstract

Dark chocolate has gained popularity in the past few years for its acclaimed health benefits such as lowering blood glucose level, lowering blood pressure and increasing dopamine levels. Preclinical studies associate dark chocolate of varying levels of cocoa contents with different dopamine levels. The present study aims to investigate the effects of consumption of dark chocolate with varied cocoa content (40%,70%,85%) on urine dopamine levels in the Islanders population. The study employs a randomized complete block design with two factors, cocoa content and dosages, and one blocking factor, age. The analysis will be conducted using two-way ANOVA with blocking. The study aims to provide insights on the potential influences of different cocoa concentrations in dark chocolate on enhancing urinary dopamine levels. By understanding the relationship between cocoa content and dopamine levels, this study could support the development of dietary recommendations for consumers with needs of increasing dopamine levels.

2 Introduction

Dopamine is a neuromodulatory molecule that plays crucial roles in humans. In the brain, several distinct dopamine pathways are involved in both physiological and behavioral processes, including movement, cognition, executive functions, reward, motivation, and neuroendocrine control. In reward-motivated behavior, the anticipation of most types of rewards increases the level of dopamine in the brain, thus controlling our feelings of well-being.

Healthy aging comes with altered dopamine functioning and is associated with reduced performance on cognitive control tasks, such as response inhibition. For instance, Parkinson's disease (PD), a neurodegenerative disorder highly prevalent among the elderly population, is believed to develop due to brain-based dopamine insufficiency (Bloemendaal et al.).

Polyphenols, reversible monoamine oxidase (MAO) inhibitors, are mood modulators that improve neuronal well-being by increasing dopamine, serotonin, and noradrenaline levels in brain tissue (Grabska-Kobyłecka et al.). Polyphenols can thus help to prevent neurodegenerative diseases and their development. Additionally, flavonoids have been experimentally demonstrated to be effective in increasing dopamine levels by protecting dopamine neurons.

Cocoa is mainly composed of polyphenols and flavonoids. Dark chocolate has a relatively high concentration of cocoa, which can be beneficial to elderly people in preventing aging-related issues when consumed in appropriate amounts. Our study aims to explore the relationship between the dosage of cocoa intake and urine dopamine levels. We hypothesize that an increase in the cocoa concentration of dark chocolate will lead to an increase in urine dopamine levels.

3 Method

3.1 Participants

The participants in the study are sampled from the Island. We employed a multi-stage sampling method with three stages. First we selected a village, then a house, and then a person. The process was repeated for 198 participants and we ended up with 99 in each age block. The gender within each age block is roughly equal but we did not use gender as a blocking factor. We used an R sample function to randomly assign people to treatment groups.

3.2 Design

This study implemented a two-way randomized complete block design. The two factors of interest are cocoa content and chocolate dosages. We are also interested in the interaction between cocoa content and dosages because we want to find the best combination of dark chocolate cocoa content and dosage to boost dopamine. The blocking factor is age of the participants, where we divided the participants into 45+ and 45- with equal numbers in each group. The reference age 45 was chosen based on literature review where it was suggested that dopamine level in human bodies decreases after the age of 45 (Knoll 57).

Response Variable	Urine Dopamine Levels		
Treatment 1 (cocoa content)	40%	70%	85%
Treatment2 (dosages)	50g	100g	150g
Blocking (Age)	45+	45-	

Figure 1: Factor Table with levels

The factor diagram is detailed below:

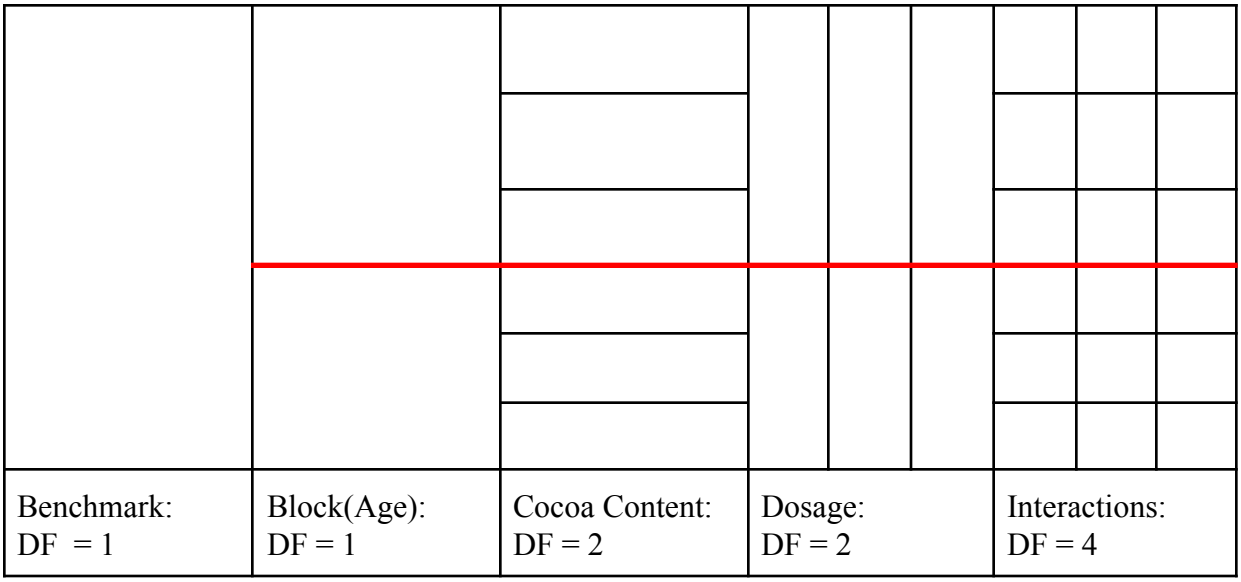

Figure 2: Factor Diagram for two-way randomized complete block design

3.3 Instruments

The urine dopamine level of subjects was measured in units of μg/hr (milligrams per hour). The dark chocolates are administered to participants, 50g per serving. Measuring dopamine levels in urine is reliable because dopamine penetrates the blood-brain barrier via monoamine transporters and is filtered in the glomerulus before entering the urine (Marc et al. 635). As a result, it can serve as a measure of total dopamine neurotransmitter state in our body (Hinz et al. 177). We measured the second urine dopamine level after an hour of the

administration of dark chocolate, assuming that the digestion and ingestion of cocoa in dark chocolate was completed within this time.

3.4 Procedure

Step 1: Find healthy and consenting Islanders using a multi-stage sampling method. 99 islanders of age 45 under and 99 islanders of over age 45.

Step 2: Randomly assign these individuals to different treatment groups using a R sample function. The different groups are:

1) Dark chocolate with 40% cocoa, 50 g
2) Dark chocolate with 70% cocoa, 50g
3) Dark chocolate with 85% cocoa, 50g
4) Dark chocolate with 40% cocoa,100g
5) Dark chocolate with 70% cocoa, 100g
6) Dark chocolate with 85% cocoa, 100g
7) Dark chocolate with 40% cocoa,150g
8) Dark chocolate with 70% cocoa, 150g
9) Dark chocolate with 85% cocoa, 150g

In each of the treatment groups there are 9 participants.

Step 3 : For each unit(islander), measure their urine dopamine.

Step 4 :For each unit, administer the dark chocolate with specific cocoa content and dosage according to the treatment groups the unit belongs to.

Step 5: For each unit, measure the urine dopamine again. This is done after an hour of administration of dark chocolate.

Step 6 : For each unit, compute the difference of dopamine level before and after administration of dark chocolate, and this difference in dopamine level will be our response variable.

4 Data Analysis

4.1 Type of statistical analysis

In our study, we will use ANOVA to analyze the data and determine whether the cocoa content in dark chocolate or the dosage affects an individual's urine dopamine levels. By performing F-tests within treatments and blocks, we aim to identify any significant differences in urine dopamine levels between the groups and examine if there is an interaction between dosage and treatment. To aid the understanding of our result we will use boxplot and interaction plots to visualize the relationship between cocoa content, dosage and urine dopamine levels. We will also plot the residual diagnostics to check the validity of our results.

4.2 Hypothesis

For the fixed-effects model with two factors (cocoa content and dosage) and interactions between the two factors, we are testing the following hypothesis:

1. $H_0: \tau_1 = \tau_2 = \tau_3 = 0$ (No main effect of cocoa content on dopamine level)

   $H_1: at\ least\ one\ \tau_i \neq 0$

2. $H_0: \beta_1 = \beta_2 = \beta_3 = 0$ (No main effect of dosages on dopamine level)

   $H_1: at\ least\ one\ \beta_i \neq 0$

3. $H_0: (\tau\beta)_{11} = (\tau\beta)_{22} = (\tau\beta)_{33} = 0$ (No interactions of cocoa content and dosage on dopamine level)

   $H_1: at\ least\ one\ (\tau\beta)_{ij} \neq 0$

4.3 Sample Size Determination

For the sample size, we used G-power to determine the sample size of our two-way randomized complete block design to be 197, where it was rounded up to 198 to balance out the numbers in each group. As a result we will have 11 participants in each of the 9 treatment

groups. With this sample size, we achieved a power of 0.8, which is the probability of rejecting the null hypothesis when it is false. For alpha we used 0.05, which is the probability of rejecting the null when it is true. We used a conservative effect size of 0.25.

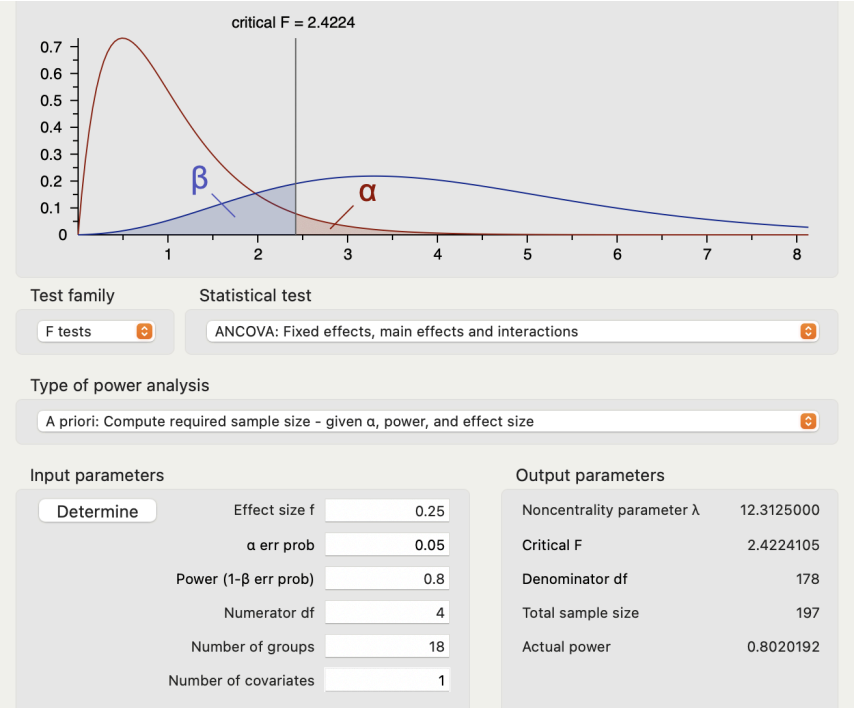

Figure 3: G power output for Sample Size Determination

5 Results

5.1 ANOVA Analysis

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age	1	0.23	0.2335	0.724	0.396
Concentration	2	0.69	0.3451	1.069	0.345
Dosage	2	0.38	0.1881	0.583	0.559
Concentration:Dosage	4	1.04	0.2607	0.808	0.522
Residuals	188	60.68	0.3228		

Figure 4:Two way ANOVA Table with Blocking and Interaction. Summarization of effects of Age, Cocoa Concentration, Dosage, and the interaction between Concentration and Dosage on

urine dopamine levels are presented in the table. Concentration, dosage, and interaction between dosage and concentration have p-value of 0.345, 0.559, and 0.522 correspondingly, suggesting that all of them do not make a statistically significant effect in change in dopamine levels.

5.2 Residual Plots

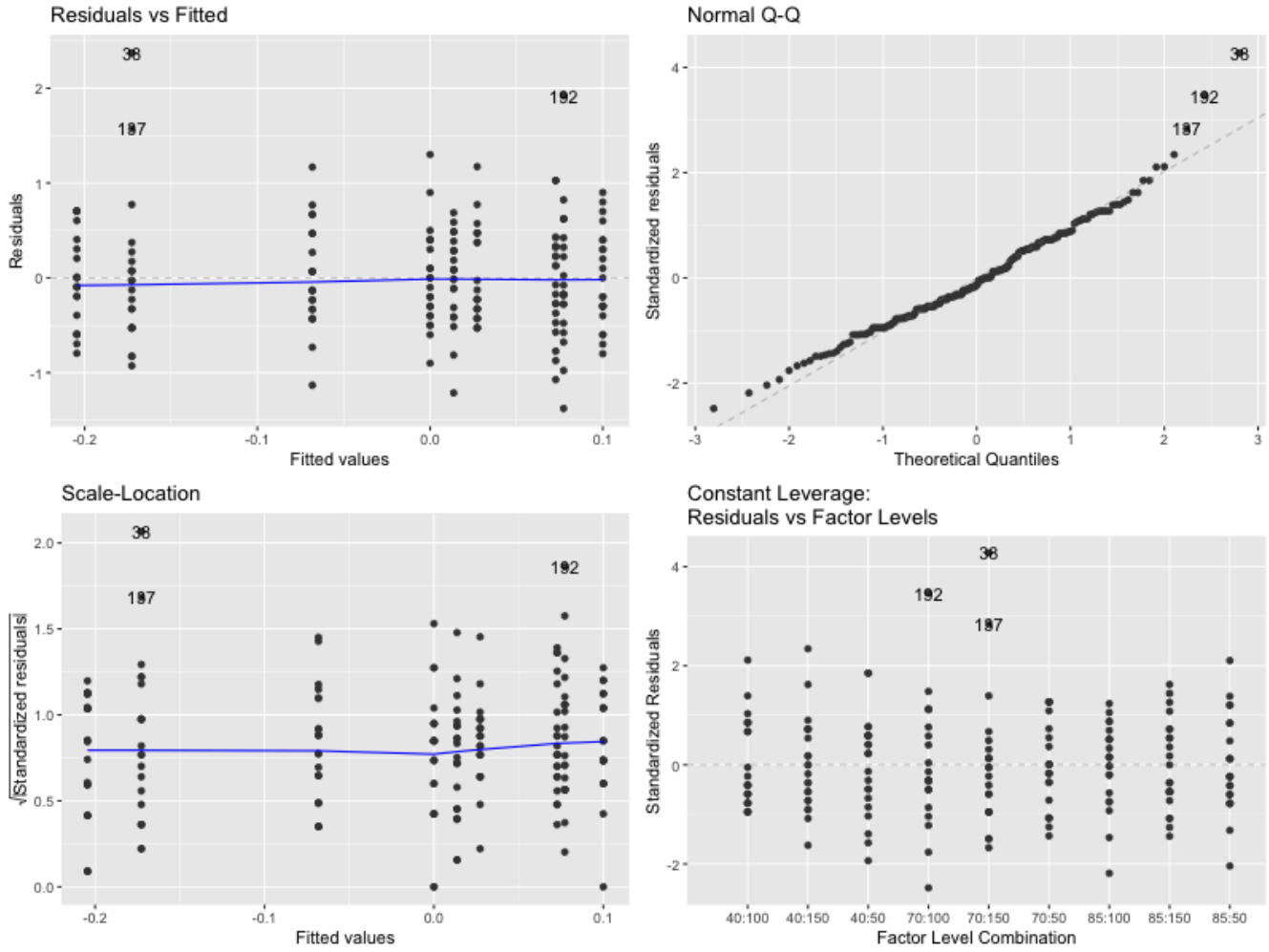

Figure 5 : Residual Plots of Model. The residual plots (Residuals vs Fitted, Normal Q-Q, Scale-Location, Constant Leverage) suggest that normality and constant variance assumption are satisfied, indicating the model is valid.

5.3 Interaction plot

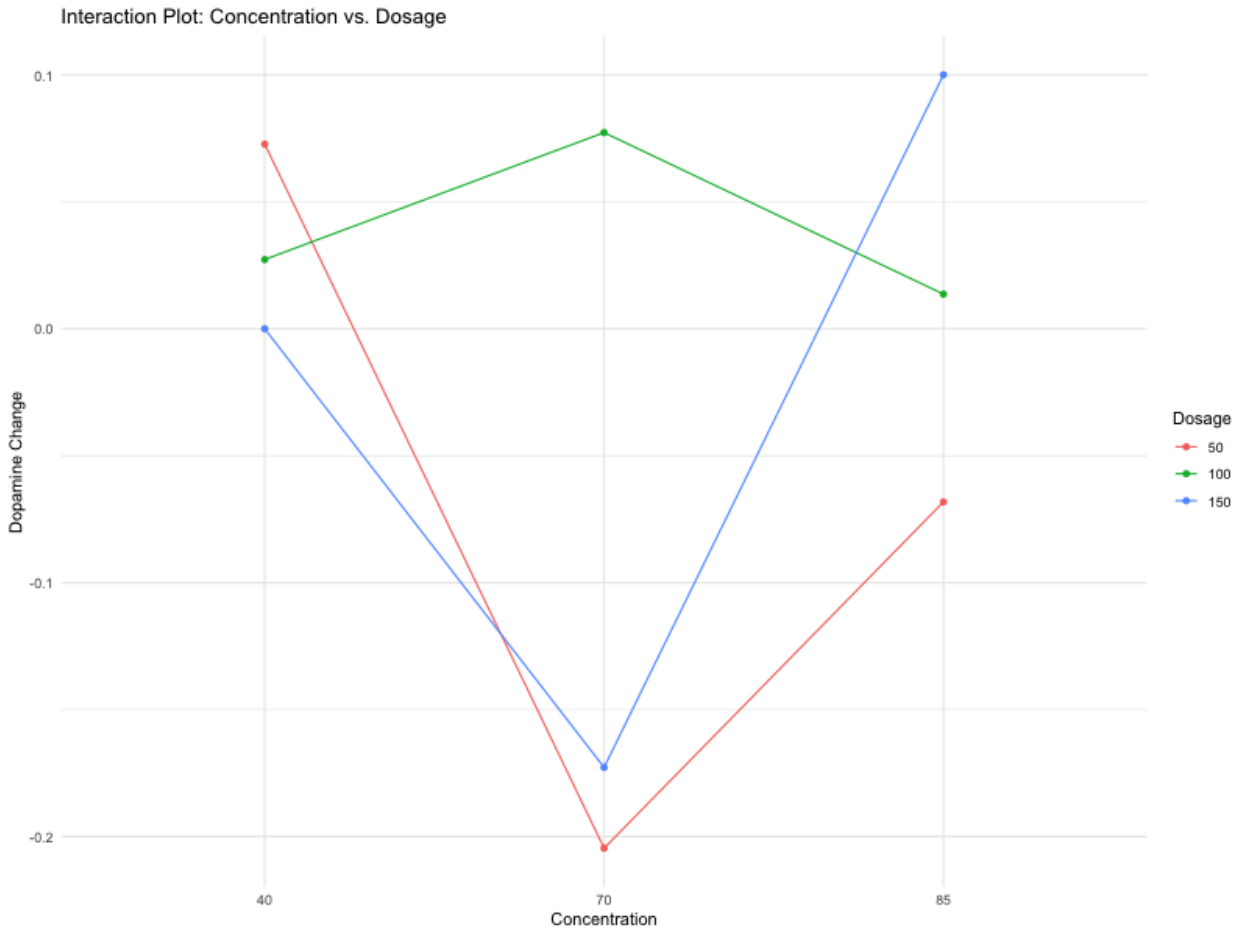

Figure 6: Interaction plot of Cocoa Concentration and Chocolate Dosage. The plot suggests that there is interaction between cocoa content and dark chocolate dosage on change in dopamine level.

5.4 Box plots

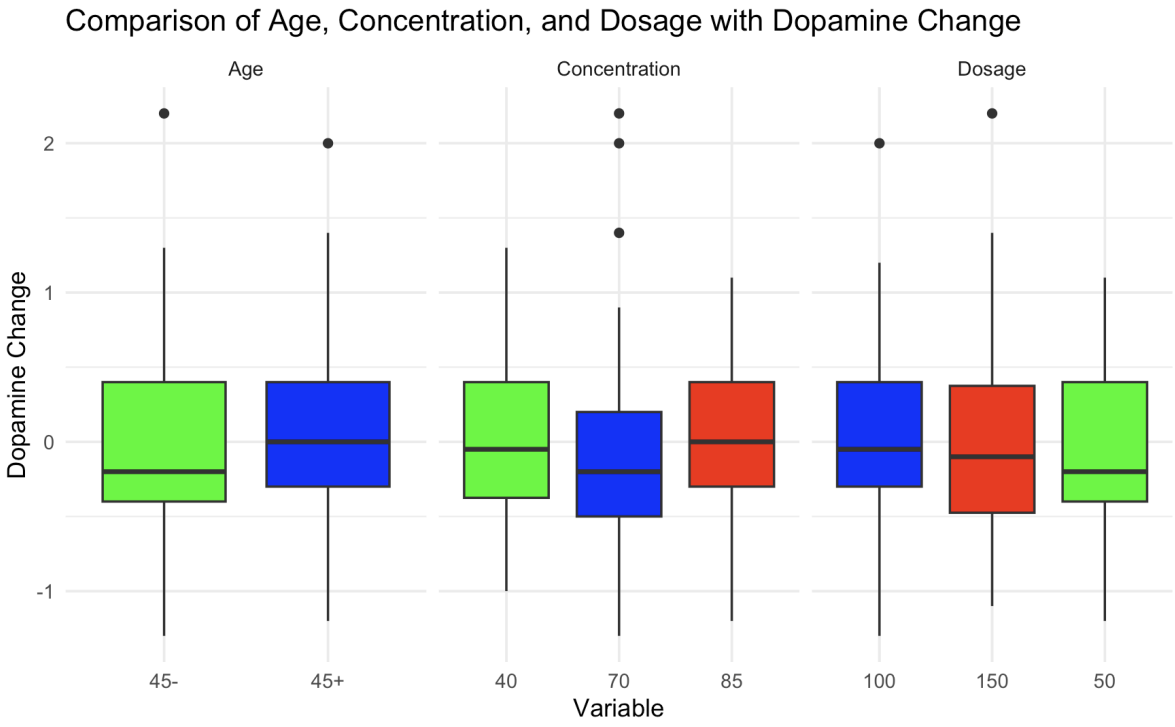

Figure 7: Side-by-side Boxplot Comparing Change in Dopamine Level between Age, Cocoa Concentration, and Dark Chocolate Dosage. The median is represented by the black bar in the middle, where the "box" depicts the 1st and 3rd quantiles. Potential outliers are displayed as black dots.

6 Discussion

Our study aims to investigate the impacts of dark chocolate with different cocoa content and chocolate dosages on urine dopamine level. The result suggests that consumption of dark chocolate with varied cocoa content and dosages of dark chocolate do not have a significant effect on urine dopamine levels.

We intended to have a sample size of 197 to achieve a power of 0.8, however, we ended up sampling 198 people in order to have a balanced number of individuals in each treatment group. After running our experiment design on Islanders, the data are processed. Since Age is used as a blocking factor, we assume no interaction between Age and the two factors, Concentration and Dosage. The results of our Analysis of Variance (ANOVA) analysis indicate that none of the factors or their interaction have a significant effect on the response variable, as all p-values are greater than 0.05. Therefore, we fail to reject the null hypothesis and conclude that: 1) cocoa content has no main effect on urine dopamine levels, 2) cocoa dosage has no main effect on urine dopamine levels, and 3) there is no interaction between cocoa content and dosage.

To check model validity, we use residual plots as diagnostic tools. The Residuals vs Fitted plot shows that the residuals are fairly randomly scattered around the horizontal line, which means linearity assumption is satisfied. Most points lie on the reference line in the Q-Q plot, suggesting that normality assumption is satisfied. In the Scale-Location plot, the residuals are evenly spread out, indicating that the variance of the errors is constant. Most points fall within the range of -2 to 2 in the leverage plot, so there are no significant issues with outliers as well. Based on these diagnostics, we conclude that our model is valid and meets the necessary assumptions for a robust analysis.

The interaction plot displays how dopamine change varies with different levels of Dosage (50, 100, and 150) across three concentration groups (40, 70, and 85), revealing the relationship between Cocoa Concentration and Dosage. Using Age as a blocking factor, we do not consider any interaction between Age and the two factors. For Dosage levels 50 and 150, there is a noticeable decrease in dopamine change at concentration of 70, followed by an increase at concentration of 85. In contrast, for Dosage level 100, the dopamine change remains relatively stable across all concentration groups, with a slight increase at concentration 85. Since the lines

intersect with each other, the interaction plot suggests that there is an interaction between cocoa concentration and dosage, and we include this effect into our model.

The boxplot reveals information about subgroups in our study. It suggests that the change in dopamine levels does not vary significantly across different categories of Age, Concentration, and Dosage. Age is used as a blocking factor in the experiment and its inclusion in the boxplot allows us to assess whether it has any noticeable effect on dopamine levels. The medians for all groups are around zero, and the spread of data is relatively similar. This observation may indicate that none of these factors have a strong influence on the change in dopamine levels in the observed data.

There are a few possible causes with the non-significant results of our study. One is with the experimental design and the other with the procedure. Our study employed a randomized complete block design, which allowed us to control the possible confounding factors such as age and reduce variability in urine dopamine levels. However, even after blocking, we see that the treatment factors are not significant, and the sum of squares of within-block error is larger than sum of squares of between-block errors. Large within-block errors indicate that person-to-person variability is high so randomized complete block design may not be the best for our study as it assumes homogeneity within each block. For future investigation on the same topic, we might want to consider a Graeco-Latin Square design where two factors of interest are cocoa contents and chocolate dosage with 5 levels each; and nuisance factors are age and individual variability. With this design we might be able to eliminate person-to-person variability as much as possible, however, it might not be very convenient to conduct this design on the Island because there is no dark chocolate with 5 different cocoa contents. An alternative is to include milk chocolate as one lower cocoa level and white chocolate as a baseline level. We did not adopt this design because based on previous literature and the available choices of actions on the Island, a two way randomized block design seemed to be sufficient.

The second part is the subtleties within the procedure of our study. First, dopamine decomposition time is unknown. Before the measurement of urine dopamine levels, we waited for 1 hour to allow chocolate to be digested and the cocoa content to be ingested into the body. However, dopamine could have been decomposed within this time delay. Second, the time taken

for dark chocolate to be digested is unknown. Thus, the time point of urine sample collection may not be ideal to have dopamine level in urine to build up. The uncertainty in the Island system complicates our procedure and could have adversely affected the results by not optimizing the effect of our treatment factor before taking measurements. Third, the sugar content in chocolate is unknown. Previous research indicates that sugar intake will lead to an increase in dopamine level (Rada et al. 737). However, during experiment, the sugar content in dark chocolates of different cocoa content is unknown and thus is not controlled. Therefore, sugar content can influence the change in dopamine level.

There are several improvements we can do to improve the experiment. First, we can introduce more variables, such as milk chocolate. Greater difference in cocoa content might lead to more significant difference in dopamine level. Second, we can improve the timing of our urine sample collection to better capture the peak dopamine levels post-chocolate consumption. Preliminary study and experiment can be conducted to determine the optimal time for dopamine measurement in urine after chocolate ingestion.

7 References

1) Bloemendaal, Mirjam et al. “Neuro-Cognitive Effects of Acute Tyrosine Administration on Reactive and Proactive Response Inhibition in Healthy Older Adults.” *eNeuro* vol. 5,2 ENEURO.0035-17.2018. 30 Apr. 2018, doi:10.1523/ENEURO.0035-17.2018
2) Grabska-Kobyłecka, Izabela et al. “Polyphenols and Their Impact on the Prevention of Neurodegenerative Diseases and Development.” *Nutrients* vol. 15,15 3454. 4 Aug. 2023, doi:10.3390/nu15153454
3) Marc, D. T., et al. "Neurotransmitters Excreted in the Urine as Biomarkers of Nervous System Activity: Validity and Clinical Applicability." *Neuroscience & Biobehavioral Reviews*, vol. 35, 2011, pp. 635-644.
4) Hinz, Marty, et al. "Neurotransmitter Testing of the Urine: A Comprehensive Analysis." *Open Access Journal of Urology*, vol. 2, 2010, p. 177.
5) Knoll, J. “Deprenyl (selegiline): the history of its development and pharmacological action.” *Acta Neurologica Scandinavica. Supplementum*, vol. 95, 1983, pp. 57-80. doi:10.1111/j.1600-0404.1983.tb01517.x.
6) Rada, P et al. “Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell.” *Neuroscience* vol. 134,3 (2005): 737-44. doi:10.1016/j.neuroscience.2005.04.043