A Study on Islanders to Determine the Effects of Dark Chocolate with Varying Cocoa Percentages on Blood Glucose

Vanda Suklar, Bo He, HongYe Zheng
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1 Abstract

This research explores the impact of dark chocolate with varying cocoa percentages (40%, 70%, and 85%) on blood glucose levels. Dark chocolate is rich in polyphenols, including epicatechin and catechin, known for their potential to influence glucose metabolism and slow glucose absorption. Previous studies suggest that higher cocoa content dark chocolate may offer better glycemic control compared to lower cocoa content variations. This study investigates the relationship between different cocoa percentage dark chocolates and blood glucose levels, considering single, double, and triple doses. In our study, we will focus on a representative sample of the islander's population using a Two-Way Randomized Block Design, employing a two-way ANOVA with blocking for analysis. The findings aim to provide insights into the potential benefits of dark chocolate in blood glucose management.

2 Introduction

Over the past few years, there has been significant interest in the potential health benefits associated with dark chocolate, especially when it contains a high proportion of cocoa. Dark chocolate is well-known for its high content of polyphenols, which possess antioxidative characteristics. Since cocoa is a source of polyphenols, it is hypothesized that the polyphenols in dark chocolate may improve blood glucose levels (Abbott, 2017). A study investigated how dark chocolate with higher cocoa content might influence insulin regulation and blood glucose levels, both of which are important for the prevention and control of diabetes.

The polyphenols found in dark chocolate include epicatechin and catechin, which have been shown to alter glucose metabolism and slow the absorption of glucose from the intestine. In in vivo studies, diabetic rat models have provided confirmation of cocoa extract/dark chocolate’s insulin-sensitizing impact (Shah, 2019). Furthermore, choosing dark chocolate with higher cocoa percentages may contribute to a more favorable glycemic response compared to dark chocolate with lower cocoa percentages, helping to mitigate rapid spikes in blood glucose levels.

Since these studies have provided evidence that dark chocolate with higher cocoa percentages is more beneficial, our study will delve deeper into the blood glucose benefits of dark chocolate, particularly with three different cocoa percentage content variations: 40%, 70%, and 85%. In our study, we will focus on the relationship between the administration of these three different cocoa percentage-containing dark chocolates and the impact on blood glucose levels when adding a single dose, two doses, and three doses of dark chocolate.

3 Methods

3.1 Participants

The participants used in this study are from the Islands (https://islands.smp.uq.edu.au/login.php). The study employed a multistage sampling method consisting of three stages: selecting a village, followed by a house, and ultimately selecting a person. We compiled a list of Islanders who met the criteria, and then we used the R sample function to randomly assign treatment to participants. Finally, we selected consenting participants who met the criteria from the villages.

3.2 Design

We implemented a Two-Way Randomized Block Design with 2 factors and 1 blocking factor. The 2 factors are 50g dark chocolate cocoa percentage and dosage of chocolate. The blocking factor is the BMI of the participants, which we divided into 2 equally sized levels: high BMI (25+) and low BMI (below 25).

Response Variable	Blood Glucose Level		
Treatment 1 (chocolate cocoa percentage)	40% cocoa	70% cocoa	85% cocoa
Treatment 2 (Dosage)	1 dark chocolate (50g)	2 dark chocolate (50g)	3 dark chocolate (50g)
Blocking (BMI)	High BMI (25+)		Low BMI (Below 25)

The factor diagram for our experiment :

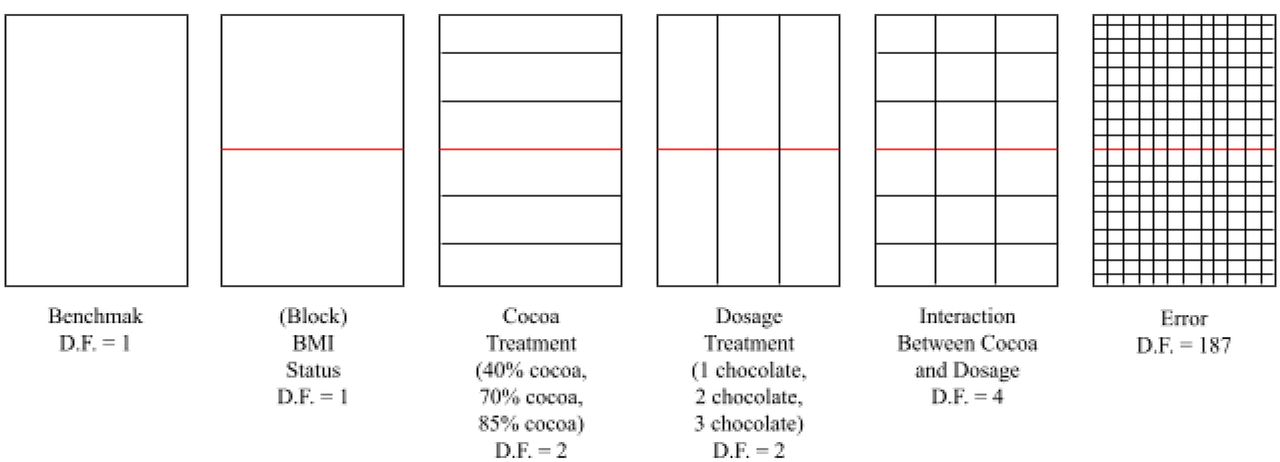


Figure: Factor Diagram for the 2-Way Randomized Complete Block Design

The 2-way randomized complete block design of mathematical model:

$y_{ijk} = \mu + \tau_i + \rho_j + (\tau\rho)_{ij} + \beta_k + \epsilon_{ijk}$
$i = 1, 2, 3$
$j = 1, 2, 3$
$k = 1, 2$
Where μ represents the mean of blood glucose, τ_i represent the effect of the ith type of cocoa dark chocolate, ρ_j represent the effect of the jth dosage, $(\tau\rho)_{ij}$ represent the interaction effect between cocoa dark chocolate type and dosage, β_k represent the effect of the the block (BMI), and ϵ_{ijk} is the error term.

3.3 Apparatus / Instruments

Blood glucose levels will be measured in the island participants using blood samples, with measurements reported in milligrams per deciliter (mg/dL). Each participant will consume three servings of dark chocolate with the same cocoa percentage (50g per serving on the island). After consuming the chocolate, their blood glucose levels will be measured to observe any changes. Previous studies have indicated that cocoa extract/dark chocolate can reduce insulin resistance (Shah, 2019). Additionally, higher cocoa percentage chocolate contains less sugar, which slows the absorption of sugar into the bloodstream (Whitaker, 2023). We assume that higher cocoa percentage chocolate will result in slower sugar absorption into the bloodstream and, consequently, better blood glucose levels compared to lower cocoa percentage chocolate. Blood glucose levels will be measured in subjects immediately before administering all substances and after each administration, for a total of three measurements.

3.4 Procedure

Step 1: Find consenting participants from the island, measure their height and weight to determine their BMI, and group them by high and low BMI.

Step 2: Within each BMI group, use R to randomly assign subjects to three different cocoa treatment groups of equal size and assign them to three dosage groups of equal size:
1. 40% cocoa dark chocolate, 1 dose
2. 40% cocoa dark chocolate, 2 doses
3. 40% cocoa dark chocolate, 3 doses
4. 70% cocoa dark chocolate, 1 dose
5. 70% cocoa dark chocolate, 2 doses
6. 70% cocoa dark chocolate, 3 doses

7. 85% cocoa dark chocolate, 1 dose
8. 85% cocoa dark chocolate, 2 doses
9. 85% cocoa dark chocolate, 3 doses

Step 3: Measure the blood glucose levels (in mg/dL) of each participant before treatment.

Step 4: Assign the treatment regimen to each participant, having them consume 40% cocoa dark chocolate, 70% cocoa dark chocolate, and 85% cocoa dark chocolate at their assigned doses (1, 2, or 3 chocolates).

Step 5: After each participant finishes a specific dose (e.g., eating one 40% cocoa dark chocolate), measure their blood glucose level. Repeat this measurement after they consume the second and third chocolates in their assigned dose group.

Step 6: Calculate the difference in blood glucose levels before and after treatment for each participant.

4 Data Analysis

4.1 Hypotheses

Null hypothesis (H0): There is no significant difference in glucose levels among individuals after consuming various cocoa content of dark chocolates and varying dosages.

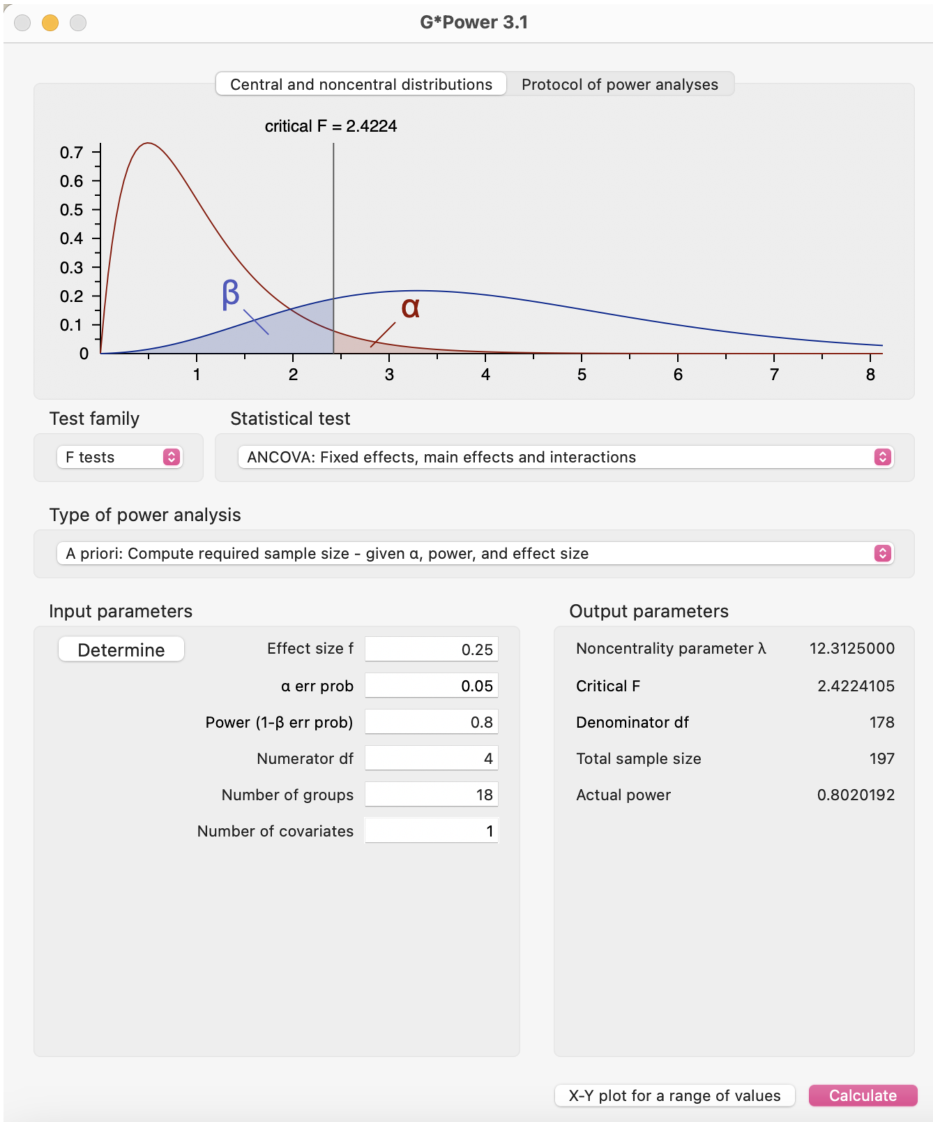
Alternative hypothesis (Ha): There is significant difference in glucose levels among individuals after consuming various cocoa content of dark chocolates and varying dosages.

4.2 type of statistical analysis

In our study, we will employ R for a comprehensive analysis. First, we will conduct a Two-Way ANOVA with Blocking and Interactions to assess the statistical significance of Cocoa Level, Dosage, and their interaction in influencing blood glucose levels. Subsequently, we will perform Post-Hoc Analysis of Treatment Types to discern mean differences across various levels. This will be followed by a Post-Hoc Analysis of Dosage to examine mean variations in blood glucose levels between different dosages.

4.3 Sample size determination

We used G*Power to determine the sample size for our experiment. For the input parameters, we utilized a conservative effect size of 0.25, which quantifies the difference between groups. We set the probability of falsely rejecting the null hypothesis (alpha) at 0.05 and the probability of correctly rejecting the false null hypothesis (power) at 0.8. The numerator degree of freedom was set to 4 (due to interaction), and we allocated 18 groups, calculated as 3 levels of cocoa content multiplied by 3 doses for each level, with 2 groups of volunteers (high vs. low BMI). Initially, our sample size estimate was 197, but for a balanced design, we rounded it to 198, with 11 samples per group.



5 Results

5.1 ANOVA Analysis:

Row	Df	Sum.Sq	Mean.Sq	F.value	Pr.F.
factor(Cocoa_level)	2	74838	37419	157.83	5.89e-41
factor(Dosage)	2	46160	23080	97.35	9.60e-30
BMI	1	2729	2729	11.51	0.000844
factor(Cocoa_level):factor(Dosage)	1	20158	5039	21.25	1.78e-14
Residuals	188	44573	237		

Table 1: Two-Way with Blocking and Interactions ANOVA table.

Based on the ANOVA table generated, the p-values of 5.89e-41, 9.60e-30 and 1.78e-14 which are all less than 0.05 suggested that variable Cocoa_level, Dosage and their interaction does make a statistically significant influence on the glucose level.

5.2 Tukey HSD Adjusted P-values

Comparison	Difference	Lower	Upper	P-value Adjusted
70 - 40	-34.46	-44.38	-24.53	7.66e-15
85 - 40	-45.70	-55.62	-35.77	< 2.22e-16
85 - 70	-11.24	-21.17	-1.32	2.20e-02

Table 2: Post-Hoc Analysis of Treatment Types.

After performing an analysis of variance (ANOVA) and finding a significant interaction effect between treatment and dosage, I used Tukey's Honestly Significant Difference (Tukey HSD) test to compare means between different levels. The change in blood glucose levels between 70% and 40% cocoa chocolate yielded a p-value of 7.66e-15. Similarly, the change in blood glucose levels between 85% and 40% cocoa chocolate resulted in a p-value of less than 2.22e16. Finally, the change in blood glucose levels between 85% and 70% cocoa chocolate had a p-value of 2.20e-02. All three comparisons showed significant differences.

Comparison	Difference	Lower	Upper	P-value Adjusted
2 - 1	12.31	1.28	23.34	2.45e-02
3 - 1	37.24	26.12	48.35	4.62e-13
3 - 2	24.93	13.86	35.99	8.57e-07

Table 2: Post-Hoc Analysis of Dosage.

After conducting Tukey's Honestly Significant Difference (Tukey HSD) test to compare the mean changes in blood glucose levels between different dosages, the following p-values were obtained: 2.45e-02 for the change in blood glucose levels between dosage 2 and dosage 1, 4.62e-13 for the change in blood glucose levels between dosage 3 and dosage 1, and 8.57e-07 for the change in blood glucose levels between dosage 3 and dosage 2. Therefore, all of these comparisons show significant differences.

5.3 Box plots

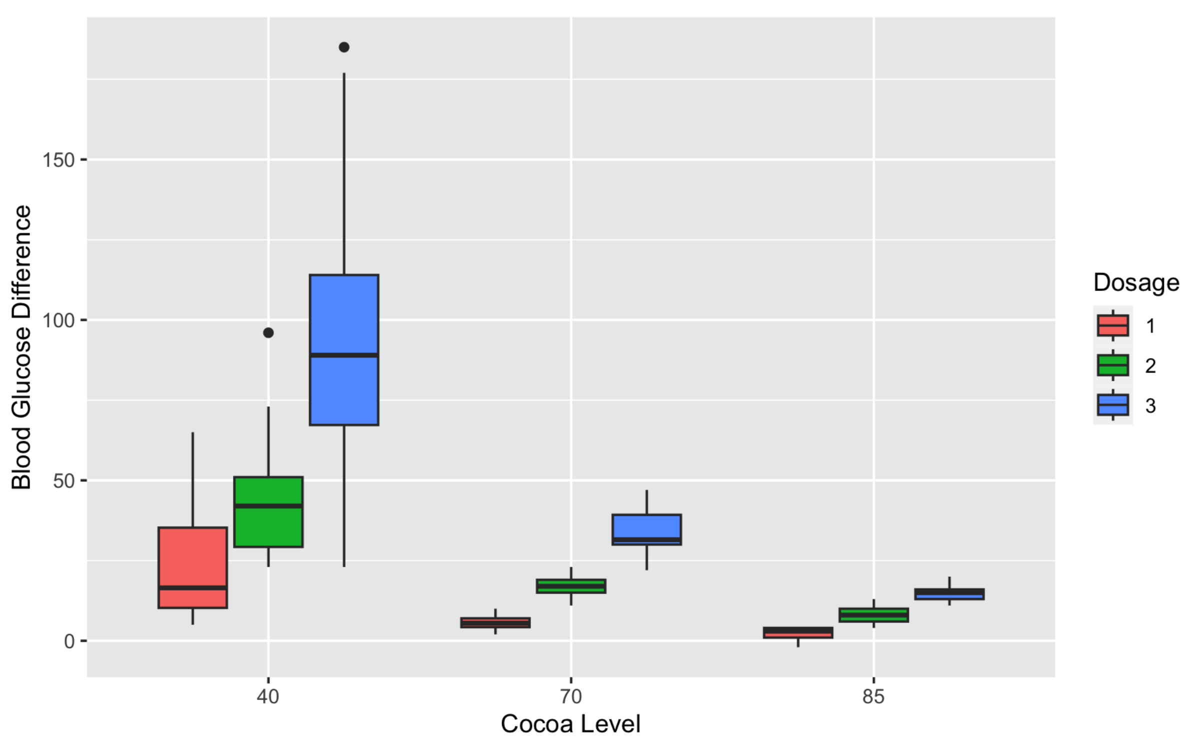


Figure 1: Side by Side Boxplot - Glucose Difference per Treatment / Dosage

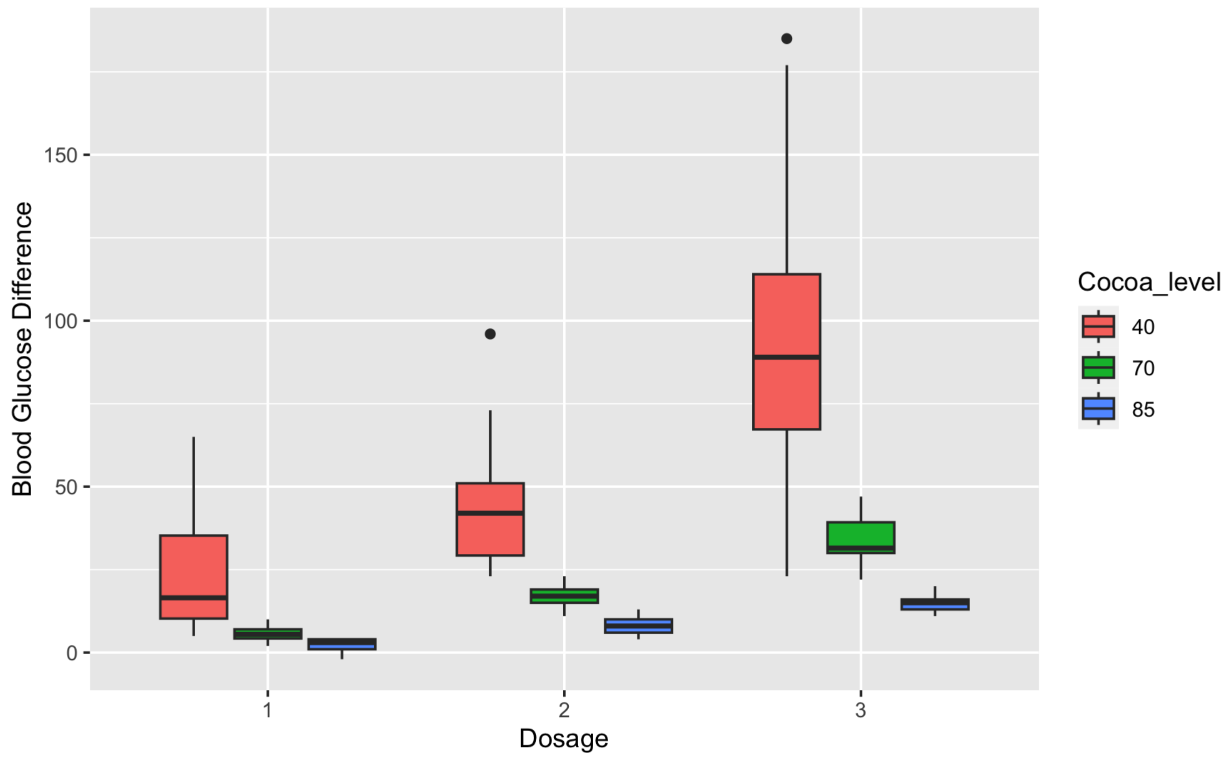


Figure 2: Side by Side Boxplot - Glucose Difference per Dosage / Treatment

In the side-by-side boxplot, the median is represented by the black line in the middle of each box, which depicts the first and third quartiles. The black dots represent potential outliers. Figure 1 shows the relationship between cocoa level and blood glucose difference. It is evident that chocolate with a 40% cocoa level has the highest blood glucose difference compared to those with 70% and 85% cocoa levels. Conversely, chocolate with an 85% cocoa level exhibits the lowest blood glucose difference. Figure 2 shows the relationship between dosage and blood glucose difference. It can be observed that a dosage of 3 has the highest blood glucose difference, while a dosage of 1 exhibits a slightly lower blood glucose difference than a dosage of 2.

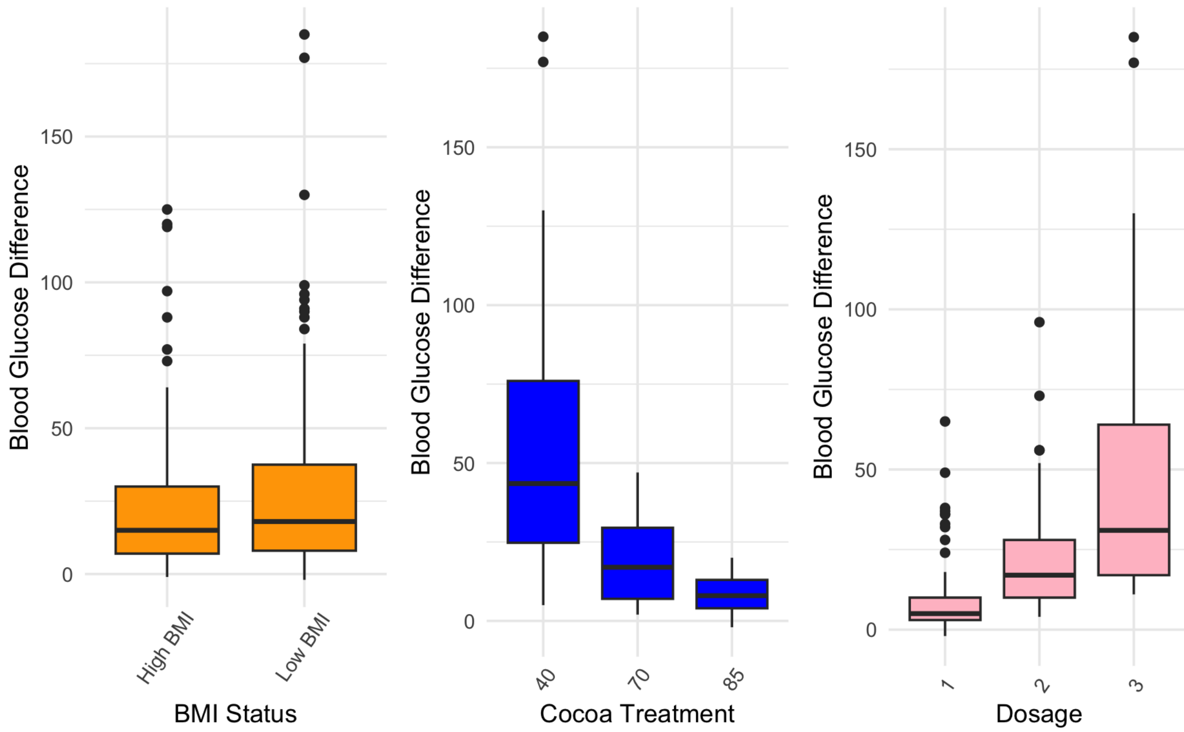


Figure 3: Boxplots comparing change in Blood Glucose between BMI Status, Cocoa Treatment, and Dose.

For these three combined boxplots, the median is represented by the black line in the middle of each box, which depicts the first and third quartiles. The black dots represent potential outliers. The first boxplot represents BMI Status vs. blood glucose difference, and we can observe that the median for low BMI is higher than that for high BMI. In the second box plot, which represents Cocoa Treatment vs. blood glucose difference, 40% cocoa chocolate exhibits the highest median blood glucose difference, while 85% cocoa chocolate has the lowest. The third boxplot represents dosage vs. blood glucose difference, with 3 dosage levels showing the highest median blood glucose difference and 1 dosage level having the lowest.

5.4 Residual Diagnostics

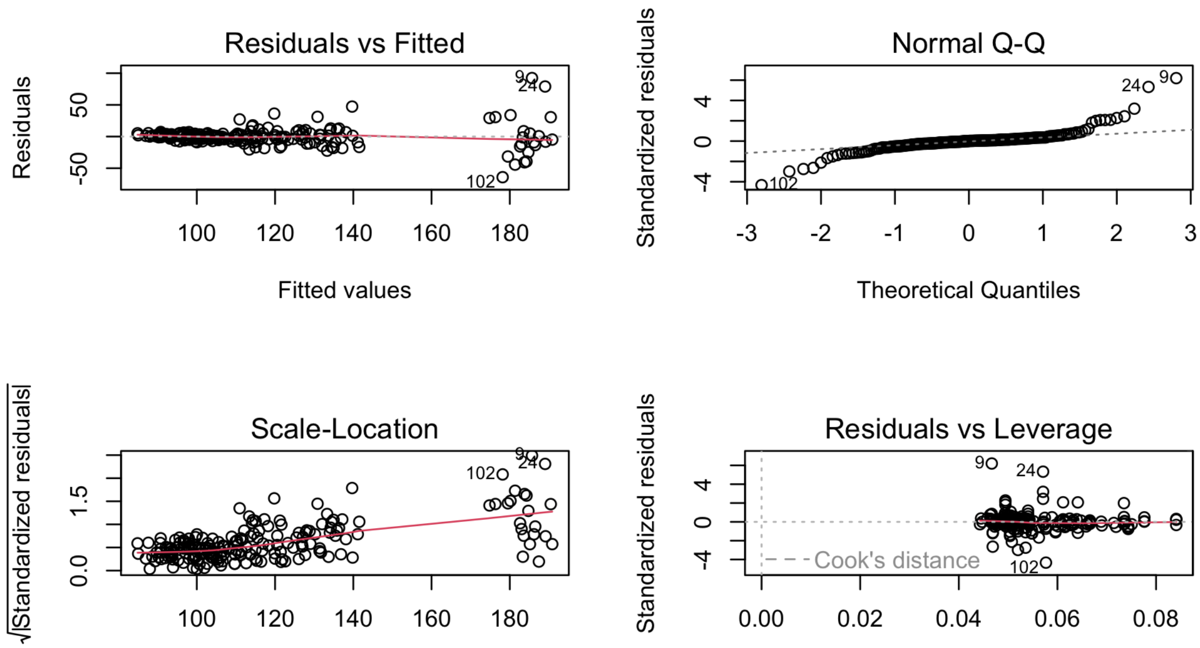


Figure 4: Residual Plots of Model

By running the residual diagnostics of the model, four plots are generated. Based on the Residuals vs Fitted plot, the red line is linear showing that the relationship is linear and the average of the residuals term is 0. Based on the Normal Q-Q plot, the points are aligned to the straight line, which implies the normality of the residuals. Based on the sqrt standardized residuals vs fitted value plot, it does not show any patterns among the points, indicating the constant variance. Based on the Residuals vs Leverage, there are no outliers. According to the four plots generated, model assumptions such as linearity, constant variance and normality are satisfied.

5.5 Interaction plot

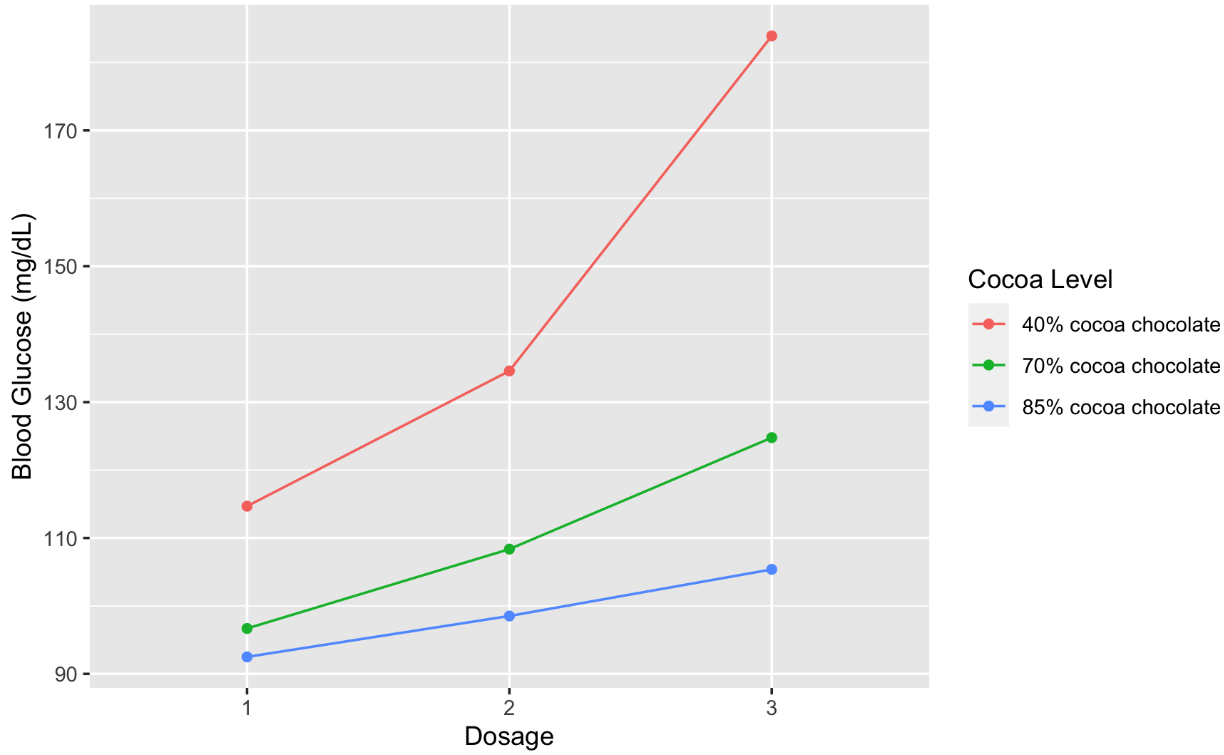


Figure 5: Interaction plot - Between Treatments and Dosage

Based on the Interaction plot generated, there are no interactions between different percentages of cocoa level and the dosage.

6 Discussion

The primary object of the study was to explore the influence of dark chocolate with various cocoa percentages on blood glucose levels. Upon previous research, the study aimed to determine whether dark chocolate with different cocoa levels content can potentially substitute/serve as a beneficial dietary supplement for people with concerns about blood glucose regulation. The initial null hypothesis stated that there would be no significant difference in glucose levels among individuals after consuming various cocoa content of dark chocolates and varying dosages. However, after performing various types of data analysis, the study rejected the null hypothesis. Therefore, the result of the study supported the idea that different cocoa percentages in dark chocolate and to some degree the dosage influenced blood glucose levels.

The study intended to have a sample size of 197 in order to achieve a power of 0.8, however the study ended up with a sample size of 198. The study first loaded the data into the R-studio and created a linear model, then by running the residuals diagnostic of the model, which satisfied the model assumptions such as linearity, homoscedasticity and normality. Then, the study runs an ANOVA analysis and based on the p-values generated, variables such as cocoa_level, dosage and their interaction which had a p-values of 5.89e-41, 9.60e-30, 1.78e-14, respectively, indicating statistically significant influence on the glucose level. The study implemented a Two-Way Randomized Block Design with two factors of cocoa_level and dosage, and blocking factor as the BMI of the participants, which were divided into two equal groups: high BMI (25+) and low BMI (below 25), controlling the source of variability for a greater accuracy of the result.

Moving further into the analysis, the ANOVA table has confirmed the variables are statistically significant. The Tukey's Honestly Significant Difference (Tukey HSD) test revealed significant differences in blood glucose levels when comparing various cocoa percentages in dark chocolates. A comparison between 70% and 40% cocoa resulted in a p-value of 7.66e-15, for 85% and 40% cocoa percentage, the p-value is is less than 2.22e-16, and comparing 85% to 70% which had a p-value of 2.20e-02. When running a Tukey HSD test to compare the mean changes in blood glucose between different various dosages, the results were: a p-value of 2.45e-02 between dosages 2 and 1, 4.62e-13 between dosage 3 and 1, and 8.57e-07 between dosage 3 and 2. All these differences were found to be significant.

The box-plots reveal the relationship between the percentage in cocoa level and blood glucose difference. In the first side by side boxplot, the study found that dark chocolate with 40% of cocoa content resulted in the highest blood glucose difference in participants compared to chocolates with 70% and 85% cocoa contents. In the second box plot, which shows the relationship between dosage and blood glucose difference, in which the study found that a dosage of 3 dark chocolate resulted in highest blood glucose difference, while dosage of 1 dark chocolate resulted in the lowest changes in the participant's blood glucose difference. In the third boxplot, which reveals the relationship between blood glucose, BMI status, cocoa level and dosage. Participants with low BMI status reflected a greater change in blood glucose difference compared to participants with high BMI status, and that participants that consumed dark chocolates with 40% of coca content and 3 doses resulted in a high blood glucose difference. Based on the boxplots that were generated, it supported the idea that the percentage of cocoa levels and degree of dosage of dark chocolates significantly influence the blood glucose levels.

In the interaction plot which reveals the relationship between the dosage and different cocoa levels, as the lines are not parallel which indicates that an interaction has occurred. This agrees with the generated ANOVA table that the p-value for the interaction between dosage and coca levels was significantly less than 0.05.

However, like all the research and experiments, the study had its constraints. The specific metabolic profiles of the participants such as their overall dietary habits, and the composition of the last meal they had, etc, which weren't controlled for and could potentially have an impact on the blood glucose measurements. Future experiments could be improved by strictly controlling these variables. Furthermore, since the consumption of dark chocolates has multifaceted effects, future studies can potentially explore its impact on inflammation, gut health and oxidative stress, etc. In summary, the study aimed to explore the influence of the consumption of dark chocolates with different cocoa percentage and dosage on participant's blood glucose. The study reached a conclusion that various cocoa percentages in dark chocolate and the rate of doses significantly influence one's blood glucose levels.

7 References

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