Nutrition Bar Effects on Blood Glucose Levels

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1 - PROJECT DESCRIPTION

For this analysis, we are testing the effect of three different nutrition bars on blood glucose levels. The data was collected previously, where twelve adults with normal blood glucose levels fasted for four hours, and then ate one of three randomly assigned nutrition bar brands. The change in their blood glucose levels compared to their fasting level was measured at 15, 45, and 75 minutes after eating. Implementing a repeated measures mixed-effects model and a linear regression, we answer the four research questions highlighted below.

1.1 - RESEARCH QUESTIONS

Question 1: Is gender important, with respect to average blood glucose levels in this experiment?
Question 2: From time periods 15 to 45 minutes, which bar(s) produce the largest average change in blood glucose levels?
Question 3: At 75 minutes, is there a difference in average blood glucose levels between any of the three bars?
Question 4: Do any of the three bars keep blood glucose levels stable across all three time periods (15, 45 and 75 minutes)?

1.2 - VARIABLES

Among the 12 participants studied, the four variables in Table 1.1 were recorded. While glucose levels were recorded prior to consumption, they were not included in this dataset. For all four research questions, the change in blood glucose level is the primary variable of interest (the response variable).

Table 1.1: Variables measured for each participant.

Variable	**Meaning**	**Values**
Gender	Gender of the participant	Male or Female
NutritionBar	Nutrition bar brand eaten by patient	BrandX, BrandY, BrandZ
Minutes	Minutes after eating the nutrition bar	15, 45, 75 minutes
Glucose	Change in glucose per deciliter of blood (mg/dl) compared to pre-consumption	Integers

Since only changes in the blood glucose levels were provided, and not the exact blood glucose levels for any of the participants or times, research questions 1 and 3 require the assumption that differences in average change in glucose levels are equivalent to differences in average glucose levels. We feel this assumption is reasonable, as all participants have normal blood glucose levels.

2 - EXPLORATORY DATA ANALYSIS (EDA)

There are a total of 36 observations, 3 for each participant, with no missing values. In order to gain preliminary insights into our research questions, we visualize the distribution of blood glucose changes subset by gender, brand, and minutes. Figure 2.1 shows 2 boxplots of blood glucose level changes, one for females and another for males, which can be used to explore research question 1. If blood glucose levels differ between males and females, we expect the boxplot median and range of values spanned to be unequal; however, we observe that this is not the case, suggesting that gender is not important in regards to average blood glucose levels.

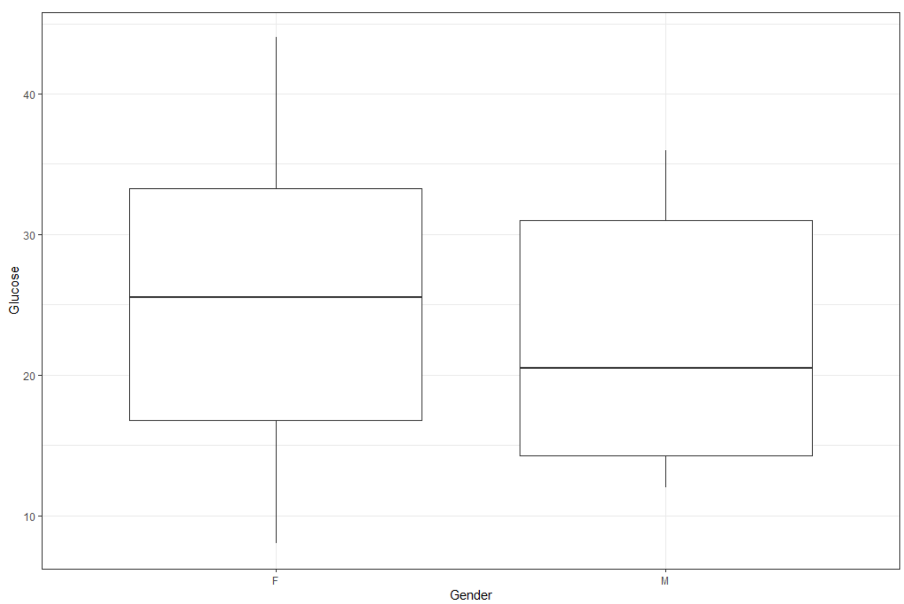

Figure 2.1: Distribution of blood glucose level changes, by gender

Figure 2.2 displays the change in blood glucose levels from 15 to 45 minutes by nutrition bar brand, which helps answer research question 2. From the figure, Brand X has the largest average blood glucose level change from 15 to 45 minutes, followed by Brand Z and then Brand Y. Brand X and Brand Z have some overlap, but the 25th percentile of Brand X changes is larger than the 75th percentile of Brand Z changes, reinforcing our conclusion. Additionally, the distribution of Brand Y does not overlap with Brand X or Brand Z, making it clear that Brand Y produces the lowest average change in blood glucose levels.

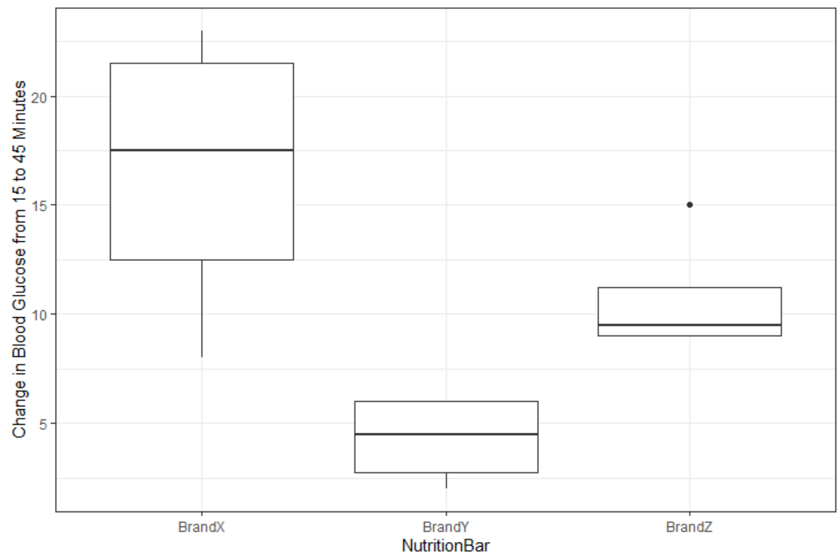

Figure 2.2: Change in blood glucose levels from 15 to 45 minutes, by brand

Figure 2.3 displays the changes in blood glucose levels by minutes within each nutrition bar brand, which gives insights into research questions 3 and 4. The blue boxplots show the distributions by brand of glucose level changes at 75 minutes, and we observe that Brand X has the largest change, followed by Brand Z and then Brand Y; this can be concluded by observing the median and the 25-75 percentile span of each boxplot. Finally, we observe that for Brand Y, the medians and 25-75 percentile spans are similar or overlap, suggesting that this brand keeps blood glucose levels stable across time. The other two brands do not have similar occurrences.

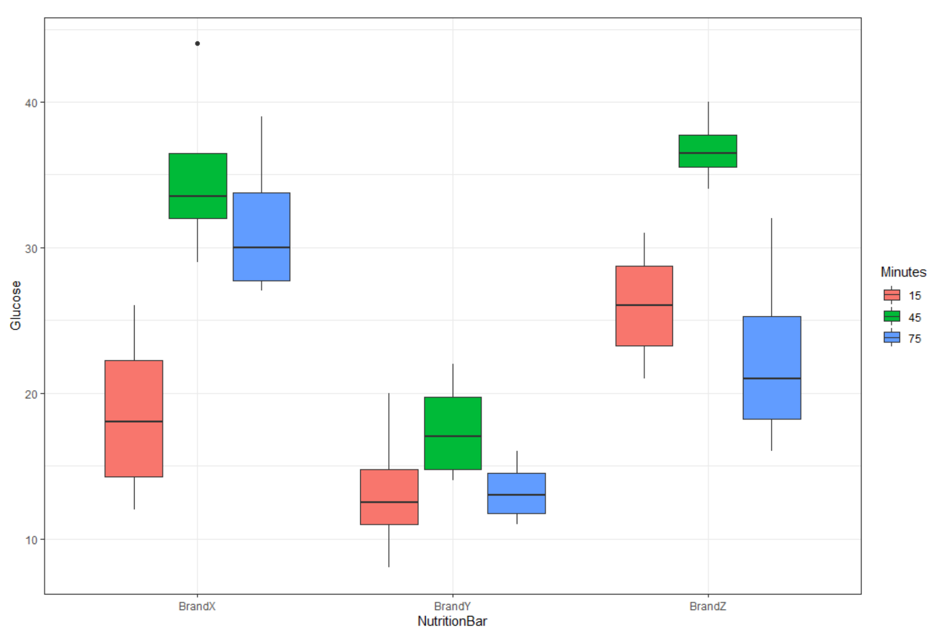

Figure 2.3: Distributions of blood glucose level changes across nutrition bar brands, grouped by minutes

3 - STATISTICAL ANALYSIS

3.1 - METHODOLOGY

Since there are both fixed and random terms considered with participants' blood glucose levels measured at multiple times, a repeated measures mixed model was chosen. To determine the best model, we first consider which terms are random, fixed, and nested. The nutrition bar brands, each participant's gender, and the three different time intervals are considered to be fixed, as they are all factors with specific given levels of interest. On the other hand, the participants themselves are random variables, as they were simply selected in a random process from a larger population of subjects. In other words, this study is not necessarily concerned with the responses of these specific participants, but rather in the overall participant response itself. The participants are nested within the larger brand variable, as each participant only receives one nutrition brand. For further explanation of a repeated measures model, fixed vs. random variables, and nested vs. crossed variables, please refer to our Resources section.

We then perform variable selection with backwards elimination, setting an alpha level of 0.05 as the selection criteria. We first start with all variables and interactions in the model, where the probability of obtaining these estimated effect sizes for each predictor is given. Based on each predictor's probability value, we slowly remove those considered to be insignificant until we arrive at a final model containing only significant predictors.

To answer question 2, we fit a linear regression model of the participant's change in blood glucose level from 15 to 45 minutes on the nutrition bar brand. This model will give definitive conclusions on whether nutrition bar brands produce different changes in blood glucose level between the two times.

Finally, to test the differences between blood glucose levels for brands at given times, we applied Tukey's multiple comparisons test to determine whether there is a significant difference between blood glucose levels of each pairwise combination of nutrition bar brands at given times to analyze whether the associated changes in blood glucose levels with each given brand/time combination is significantly different from each other.

3.2 - RESULTS

The final repeated measures mixed model includes the nutrition bar brand, the participant, the time (minutes), and the interaction between the nutrition bar brand and time (minutes), as seen below in Table 3.1. This suggests that gender is not a significant predictor, and further confirms that the relationship between the nutrition bar brand and change in blood glucose levels does indeed depend on how much time has passed. This significant interaction also confirms that the pattern of change over time differs for the nutrition bar brands, meaning that the brands and time should not be evaluated individually.

Table 3.1: Model Results

Error: Participant

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
NutritionBar	2	1503.3889	751.69444	16.27893	0.0010237
Residuals	9	415.5833	46.17593	NA	NA

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Minutes	2	696.2222	348.11111	23.675063	0.0000091
NutritionBar:Minutes	4	397.7778	99.44444	6.763224	0.0016612
Residuals	18	264.6667	14.70370	NA	NA

To answer research question 2, we use the regression model output shown in Table 3.2. In this model, since Brand X is set as the baseline, the coefficients for the other brands denote the difference in average change in blood glucose levels from 15 to 45 minutes compared to Brand X. While the negative coefficients indicate that Brands Y and Z both have lower changes in glucose level compared to Brand X, only Brand Y is significant (with a *p-value* < 0.05). This indicates that while we can state Brand Y had a smaller change in blood glucose levels compared to Brand X, there is no statistical difference betweens Brand X and Z; Brands X and Z produced equally large changes in glucose levels (with a *p-value* < 0.05).

Table 3.2: Model of changes in blood glucose level from 15 to 45 minutes, by brand

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	16.50	2.226732	7.409964	0.0000406
NutritionBarBrandY	-12.25	3.149074	-3.890032	0.0036748
NutritionBarBrandZ	-5.75	3.149074	-1.825934	0.1011465

We apply Tukey comparisons in Table 3.3 to investigate whether the different interactions between time (minutes) and the nutrition bar brands are considered significant. In other words, we inspect to see which relationships between changes in blood glucose levels and nutrition bar brands may be reliant on time enough to warrant inclusion in the model. We particularly find that for 75 minutes, Brand X produces different average blood glucose levels than the other two brands, since it does not share a group with these brands. Additionally, Brand Y shares a group at all 3 times, suggesting that this brand keep blood glucose levels stable.

Table 3.3: Tukey pairwise comparisons for interaction between brand and minutes

NutritionBar	Minutes	Mean	SE	DF	Lower CL	Upper CL	Group
BrandY	15	13.25	2.509703	20.04807	8.015655	18.48434	A
BrandY	75	13.25	2.509703	20.04807	8.015655	18.48434	A
BrandY	45	17.50	2.509703	20.04807	12.265655	22.73434	AB
BrandX	15	18.50	2.509703	20.04807	13.265655	23.73434	AB
BrandZ	75	22.50	2.509703	20.04807	17.265655	27.73434	ABC
BrandZ	15	26.00	2.509703	20.04807	20.765655	31.23434	BCD
BrandX	75	31.50	2.509703	20.04807	26.265655	36.73434	CDE
BrandX	45	35.00	2.509703	20.04807	29.765655	40.23434	DE
BrandZ	45	36.75	2.509703	20.04807	31.515655	41.98434	E

Additionally, we explore Tukey comparisons of time and nutrition bar brands individually in Tables B.1 and B.2. These comparisons find differences in average blood glucose level between brands and time. A more thorough explanation of how to interpret Tukey pairwise comparisons is provided in the Resources section.

3.3 - DIAGNOSTICS

The results in Section 3.2 may be misleading if we do not check that we are fulfilling the assumptions of a repeated model. The assumptions are that the test variables follow a multivariate linear relationship, the observations are independent, sphericity should show that the pairwise differences in variance between the samples are equal, and the responses follow a normal distribution for each level of the within-subject factors (nutrition bar brand). As shown in Table B.1, all the assumptions are met for each test area. An explanation of the assumptions can be found in the Resources section.

4 - RECOMMENDATIONS

Question 1: *Is gender important, with respect to average blood glucose levels in this experiment?*
After controlling for brand and time, gender is not important with respect to average blood glucose levels in this experiment.

Question 2: *From time periods 15 to 45 minutes, which bar(s) produce the largest average change in blood glucose levels?*

From time periods 15 to 45 minutes, the Brand X nutrition bar and the Brand Z nutrition bar equally produce the largest average change in blood glucose level. Brand Y does not produce a statistically significant average change in blood glucose levels during this time period.

Question 3: *At 75 minutes, is there a difference in average blood glucose levels between any of the three bars?*
At 75 minutes, the Brand X nutrition bar produces a higher average blood glucose level than the Brand Y nutrition bar. There is no statistically significant difference between Brand X and Brand Z or between Brand Y and Brand Z.

Question 4: *Do any of the three bars keep blood glucose levels stable across all three time periods (15, 45 and 75 minutes)?*
The Brand Y nutrition bar keeps blood glucose levels stable across all three time periods, but the other nutrition bar brands vary with the time period.

5 - RESOURCES

Below are several readings which further explain the methodology used in our analysis.

- Repeated measures overview (Section 3.1)
- Understanding crossed and nested (Section 3.1)
- Distinguishing between random and fixed (Section 3.1)
- Interpreting Tukey pairwise comparisons (Section 3.2)
- Repeated measures assumptions/interpretation (Section 3.3)

6 - ADDITIONAL CONSIDERATIONS

One limitation for this study is that it has a rather low sample size, considering we have data for only 12 participants, or four for each nutrition bar brand. A low sample size reduces the power of our model, which leads to a higher probability that a statistical test will fail to identify differences among the three nutrition bar brands, if they truly exist. Thus, we must be careful about what conclusions we draw, seeing as we are analyzing a limited amount of data.

Another concern is that the data contains changes in blood glucose, not the levels, as mentioned in Section 1.2. Although all the participants have normal blood glucose levels, we cannot necessarily make definitive conclusions on the answers to research questions 1 and 3. Further studies should seek to replicate this analysis with blood glucose levels.

Thank you for the opportunity to work on this project, and we look forward to future collaborations.

Appendix A: Exploratory Data Analysis

Table A.1: Summary statistics for blood glucose levels

variable	mean	sd	min	med	max
Glucose	23.80556	9.677129	8	22.5	44

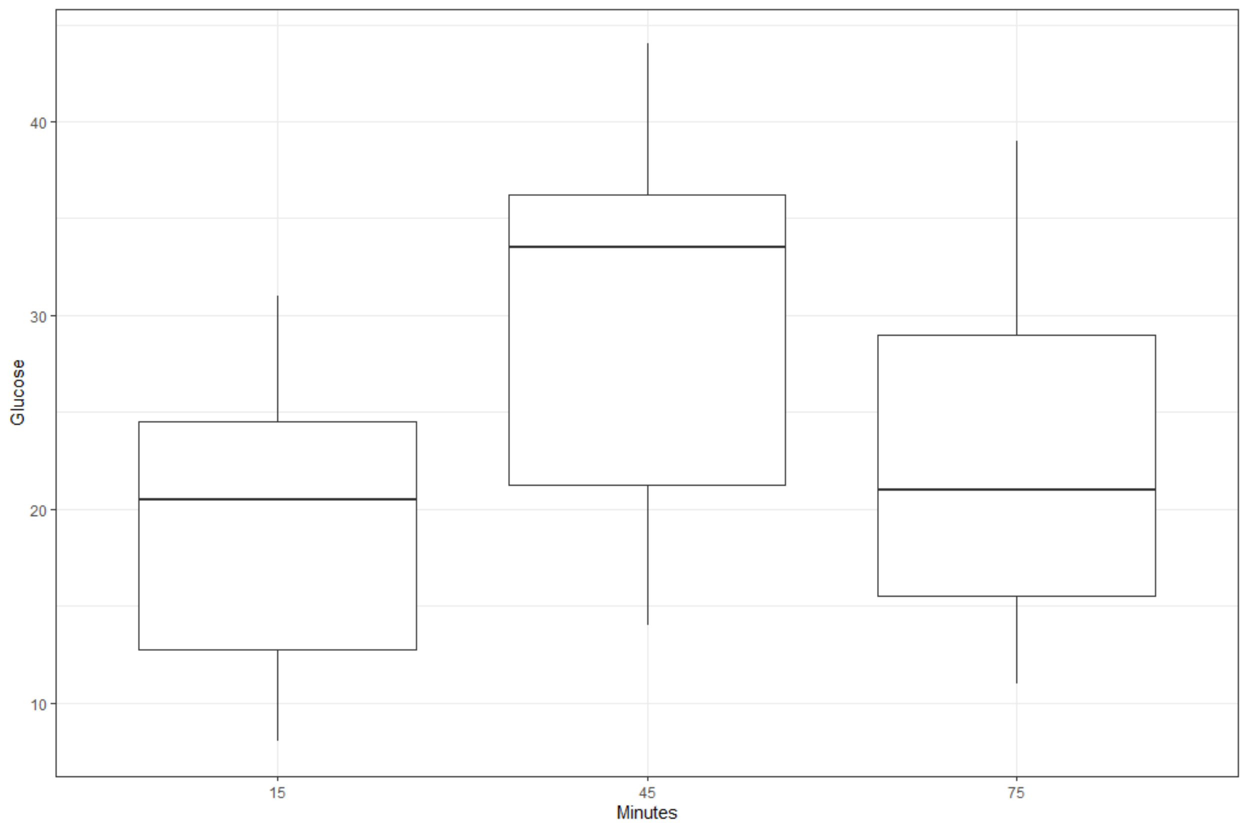

Figure A.1: Distribution of blood glucose levels, by minutes

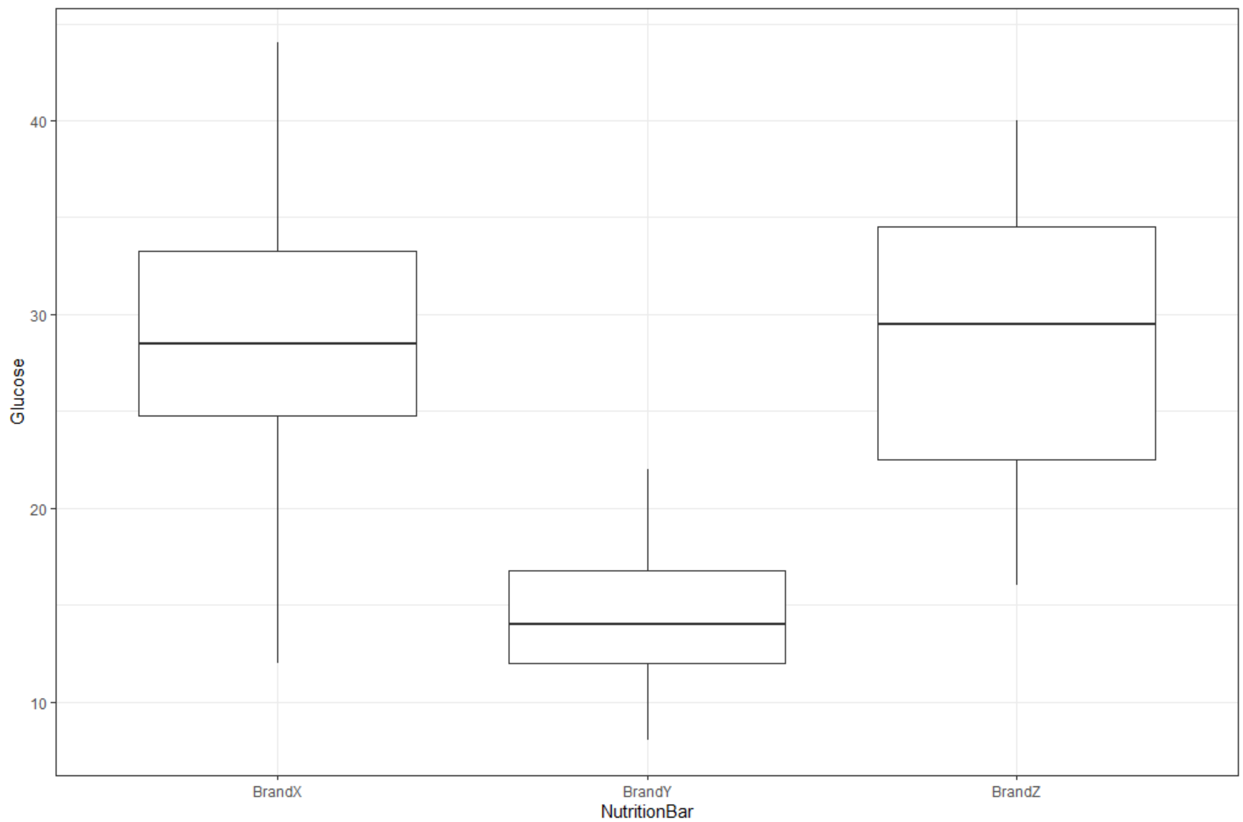

Figure A.2: Distribution of blood glucose levels, by brand

Appendix B: Models

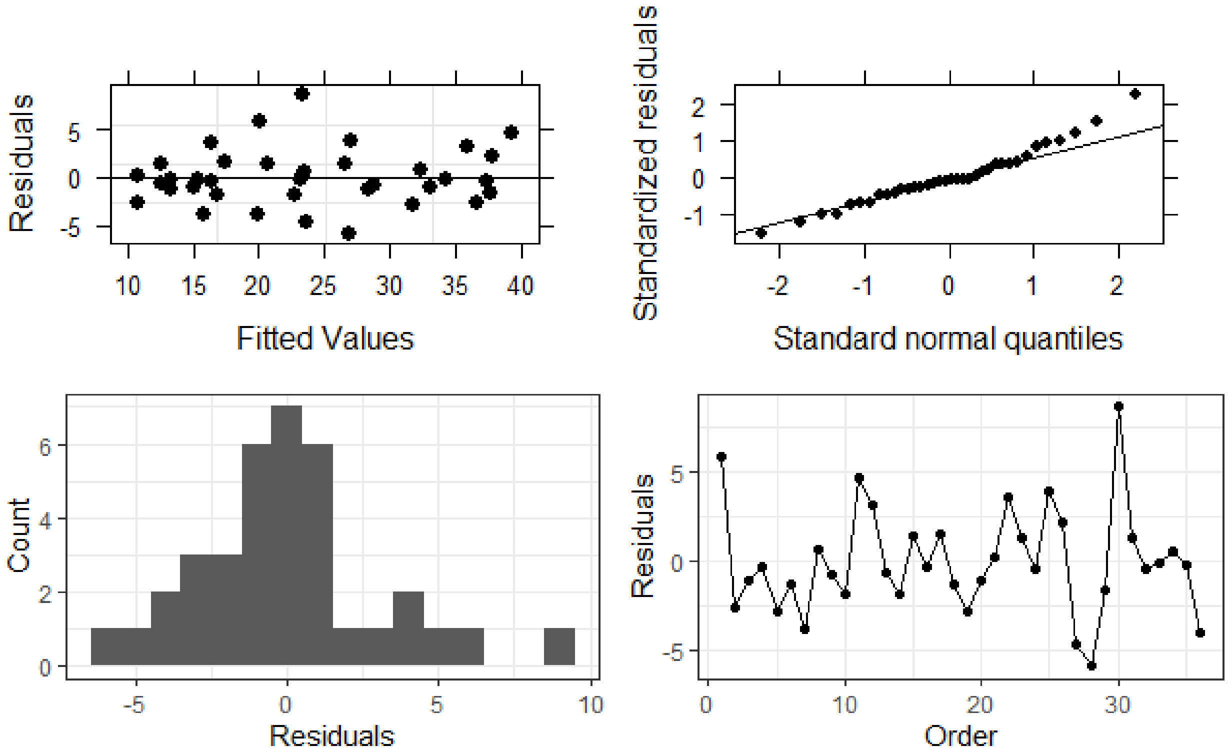

Figure B.1: Assumption plots for the final model

Table B.1: Tukey pairwise comparisons for nutrition bar brand

1	estimate	SE	df	t.ratio	p.value
BrandX - BrandY	13.6666667	2.774164	9	4.9264085	0.0021116
BrandX - BrandZ	-0.0833333	2.774164	9	-0.0300391	0.9995027
BrandY - BrandZ	-13.7500000	2.774164	9	-4.9564476	0.0020274

Table B.2: Tukey pairwise comparisons for minutes

1	estimate	SE	df	t.ratio	p.value
15 - 45	-10.500000	1.565445	18	-6.707359	0.0000079
15 - 75	-3.166667	1.565445	18	-2.022854	0.1353482
45 - 75	7.333333	1.565445	18	4.684505	0.0005161

Appendix C: R Code

Data Processing

```
# libraries
library(tidyverse)
library(data.table)

# read data and save as RDS
nb <- fread("Data/NutritionBarData.csv")
nb1 <- nb %>%
  mutate(Minutes = as.factor(Minutes),
         Participant = as.factor(Participant))
saveRDS(nb1, file = "Data/nb.rds")
```

Exploratory Data Analysis

```
# libraries
library(tidyverse)
library(emmeans)

# visual options
theme_set(theme_bw())

# load data
nb <- readRDS("Data/nb.rds")

# gender diff. in glucose
ggplot(nb, aes(x = Gender, y = Glucose)) +
  geom_boxplot()

# brand diff. in glucose
ggplot(nb, aes(x = NutritionBar, y = Glucose)) +
  geom_boxplot()

# brand stability
ggplot(nb, aes(x = NutritionBar, y = Glucose, fill = Minutes)) +
  geom_boxplot()

# minute diff. in glucose
ggplot(nb, aes(x = Minutes, y = Glucose)) +
  geom_boxplot()
```

```
# 15 to 45 change
nb1 <- nb %>%
  pivot_wider(id_cols = c(Participant, NutritionBar, Gender), names_from = Minutes,
values_from = Glucose) %>%
  mutate(Change = `45` - `15`)
ggplot(nb1, aes(x = NutritionBar, y = Change)) +
  geom_boxplot() +
  ylab("Change in Blood Glucose from 15 to 45 Minutes")

# save condensed data
saveRDS(nb1, "Data/nb_condensed.RDS")
```

Modeling

```
# libraries
library(tidyverse)
library(lme4)
library(lattice)
library(gridExtra)
library(knitr)

# load data
nb <- readRDS("Data/nb.rds")
nb_cond <- readRDS("Data/nb_condensed.RDS")

# visual options
theme_set(theme_bw())

# create model
mod_full <- aov(Glucose ~ (Gender+NutritionBar+Minutes)^2 + Error(Participant), data = nb)
tab_full <- summary(mod_full)
kable(tab_full$`Error: Participant`[[1]], caption = "Error: Participant")
kable(tab_full$`Error: Within`[[1]], caption = "Error: Within")

# reduce model
mod_red <- aov(Glucose ~ NutritionBar*Minutes + Error(Participant), data = nb)
tab_red <- summary(mod_red)
kable(tab_red$`Error: Participant`[[1]], caption = "Error: Participant")
kable(tab_red$`Error: Within`[[1]], caption = "Error: Within")
```

```
# create model/objects for residual plots
mod_plot <- lmer(Glucose ~ NutritionBar*Minutes + (1 | NutritionBar:Participant), data = nb)
mod_resid <- resid(mod_plot)
mod_fitted <- fitted(mod_plot)
nb1 <- nb %>% mutate(res = mod_resid,
                     index = 1:n())

# generate plots
r1 <- plot(mod_plot, xlab = "Fitted Values", ylab = "Residuals", pch = 19, col = "black")
r2 <- qqmath(mod_plot, pch = 19, col = "black")
r3 <- ggplot(nb1, aes(res)) +
  geom_histogram(binwidth = 1) +
  xlab("Residuals") +
  ylab("Count")
r4 <- ggplot(nb1, aes(index, res)) +
  geom_point() +
  geom_line() +
  xlab("Order") +
  ylab("Residuals")
grid.arrange(r1, r2, r3, r4, nrow = 2)

# save model
saveRDS(mod_plot, "reduced_model.RDS")

# create 15 to 45 change model
mod_2 <- lm(Change ~ NutritionBar, data = nb_cond) %>% summary()
kable(mod_2$coefficients)
```

Tukey Comparisons

```
# libraries
library(tidyverse)
library(knitr)
library(summarytools)
library(emmeans)

# load data
nb <- readRDS("Data/nb.rds")

# load model
mod <- readRDS("reduced_model.RDS")
```

```
# glucose summary
nb %>%
  dplyr::select(Glucose) %>%
  descr(stats = c("mean", "sd", "min", "med", "max")) %>%
  tb() %>%
  kable()

# tukey comparisons
minnb <- emmeans(mod, pairwise ~ NutritionBar * Minutes)
comp_tab <- multcomp::cld(minnb$emmeans, alpha = 0.05 , Letters = LETTERS) %>%
  rename(Mean = emmean,
         Group = .group,
         DF = df,
         `Lower CL` = lower.CL,
         `Upper CL` = upper.CL) %>%
  mutate(Group = trimws(Group),
         Group = ifelse(Group ==  "BCD", "  BCD", Group),
         Group = ifelse(Group ==  "CDE", "    CDE", Group),
         Group = ifelse(Group ==  "DE", "      DE", Group),
         Group = ifelse(Group ==  "E", "        E", Group),
         Group = gsub(" ", " ", Group, fixed = TRUE))

kable(comp_tab, row.names = F)
```