

Platinum Analysis Report

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2024-03-08

1 Analysis

Analysis was done using R software.

Analysis was performed for the following response variables: OGA.BWD_AL, SOS, CSOM.ACT(MEAN), CSOM.BEHAV(MEAN), CBPL.PSS, CBPL.PIS, CBPL.QOL, Actical (Sed, Light, Vig, Total Daily Activity Count).

Visit 1 was not used for analysis. Prior to statistical analysis, visits 2-7 were resolved into treatment and time. Visits 2,3,4 represent baseline, post1 and post2 for the initial treatment phase. Visits 5,6,7 represent baseline, post1, post2 for the second treatment phase.

Actical data was averaged over days to values corresponding to visits (using data column B1-T1-B2-T2).

Summary statistics including mean, median, standard deviation, minimum, Q1, Q3 and maximum of each response variable by treatment and time point were calculated. Boxplots are provided for each response variable by treatment and visit.

Mixed models were fit separately for each response variable. Treatment (A or B), Time (baseline, post1, post2), and an interaction between treatment and time were included as fixed effects. Patient was included as a random effect to account for variability between different individual dogs. Benjamini-Hochberg adjusted F-test p-values are provided to account for multiple response variables.

Pairwise comparisons were performed for response variables that showed evidence of a treatment or treatment/time difference. Tukey adjustments were made to account for multiple comparisons for pairwise comparisons by treatment.

Diagnostic plots were investigated to check model assumptions. For Actical Total Activity, we saw evidence of unequal variance. However, after trying a log transformation (to satisfy model assumptions), there was still no evidence of a treatment or treatment*time interaction. Hence we present the results on the original scale.

2 Results

2.1 Full Results

Boxplots.pdf shows boxplots for the response variable of interest by treatment and time point.

results.xlsx contains the following sheets:

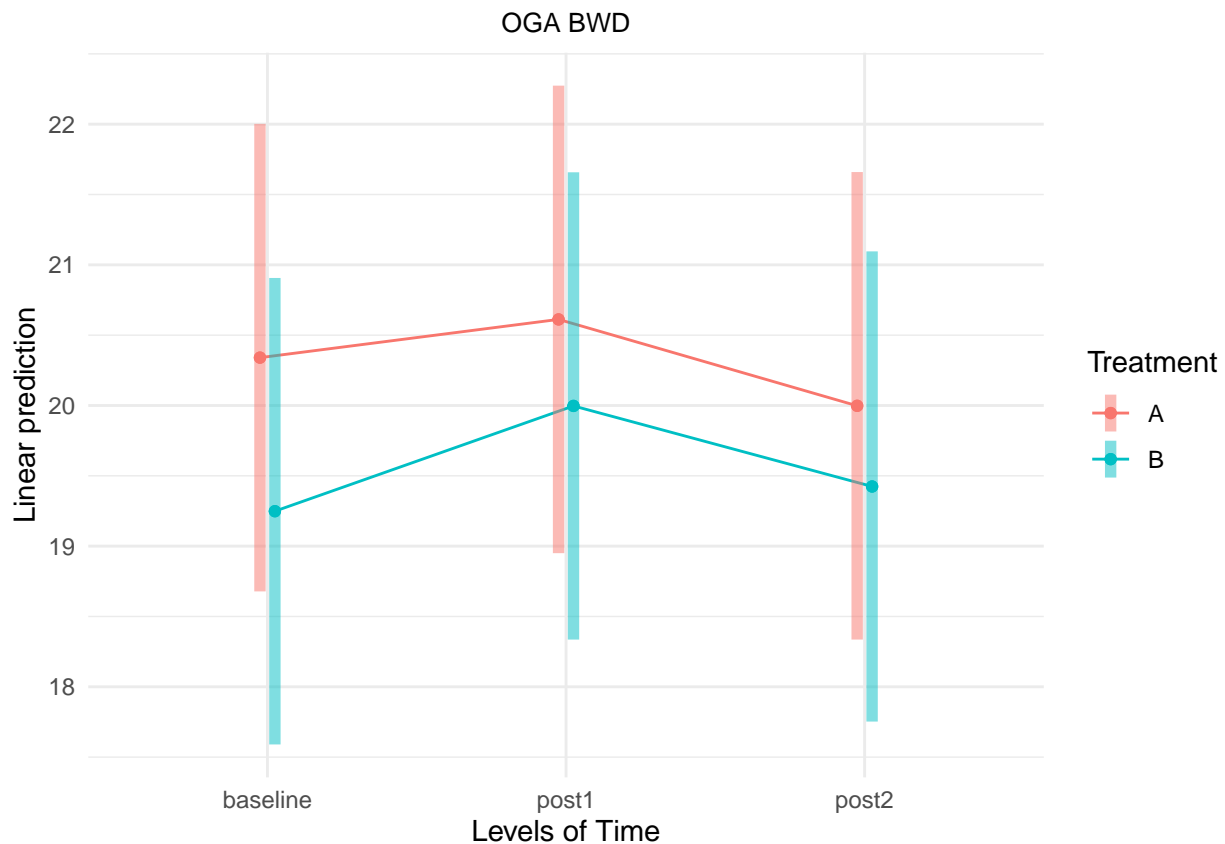
- Summary Statistics: Contains columns detailing the response variable, time, treatment, min, Q1, median, mean, Q3, max, sd, and N (sample size).

- **Mixed Model Results:** Contains columns detailing the response variable and output from the mixed model results. Raw $\Pr(>F)$ represents the unadjusted p-values and Adj $\Pr(>F)$ represents the Benjamini-Hochberg adjusted F-test p-values.
- **Pairwise Comparisons|Treatment:** Contains columns detailing output from pairwise comparisons by treatment. Only those response variables that showed evidence of a treatment or treatment*time interaction were considered for further pairwise comparisons. Tukey adjustments were made to account for multiple comparisons.
- **Pairwise Comparisons|Time:** Contains columns detailing output from pairwise comparisons by time. Only those response variables that showed evidence of a treatment or treatment*time interaction were considered for further pairwise comparisons.

For those response variables that showed evidence of a treatment or treatment*time interaction, detailed results are also provided here as well as example interpretations.

2.2 OGA BWD

2.2.1 Graph



Example Interpretation: Estimated marginal means of each treatment across time points for OGA BWD are displayed. The vertical lines represent the 95% confidence interval for the estimated marginal mean.

Table 1: Mixed Model Results

Response	Sum Sq	Mean Sq	NumDF	DenDF	F value	Raw Pr(>F)	Adj Pr(>F)
OGA.BWD_AL.Time	14.6599	7.3300	2	171.1729	2.6463	0.0738	0.2436
OGA.BWD_AL.Treatment	29.7652	29.7652	1	171.6651	10.7461	0.0013	0.0104
OGA.BWD_AL.Time:Treatment	2.9675	1.4837	2	171.1729	0.5357	0.5863	0.7987

2.2.2 ANOVA Table

2.2.3 Pairwise Comparisons by Treatment

Table 2: Pairwise Comparisons by Treatment

contrast	Treatment	estimate	SE	df	t.ratio	p.value
baseline - post1	A	-0.2718	0.3923	171.0031	-0.6930	0.7679
baseline - post2	A	0.3425	0.3923	171.0031	0.8731	0.6580
post1 - post2	A	0.6143	0.3923	171.0031	1.5661	0.2632
baseline - post1	B	-0.7484	0.3903	171.0374	-1.9176	0.1368
baseline - post2	B	-0.1758	0.4023	171.3077	-0.4369	0.9002
post1 - post2	B	0.5726	0.4044	171.2882	1.4161	0.3349

Example Interpretation: For OGA BWD A baseline - A post1: the estimated difference between means is -0.2718. Because the difference is negative this indicates that A baseline had a lower mean than A post1. (This can be confirmed using the graph or summary statistics). The corresponding Tukey-adjusted p-value is 0.7679. Recall that small p-values (typically less than 0.05) provide evidence of a difference.

2.2.4 Pairwise Comparisons by Time

Table 3: Pairwise Comparisons by Time

contrast	Time	estimate	SE	df	t.ratio	p.value
A - B	baseline	1.0917	0.3931	171.3352	2.7771	0.0061
A - B	post1	0.6152	0.3966	171.3824	1.5513	0.1227
A - B	post2	0.5735	0.4033	171.1222	1.4220	0.1568

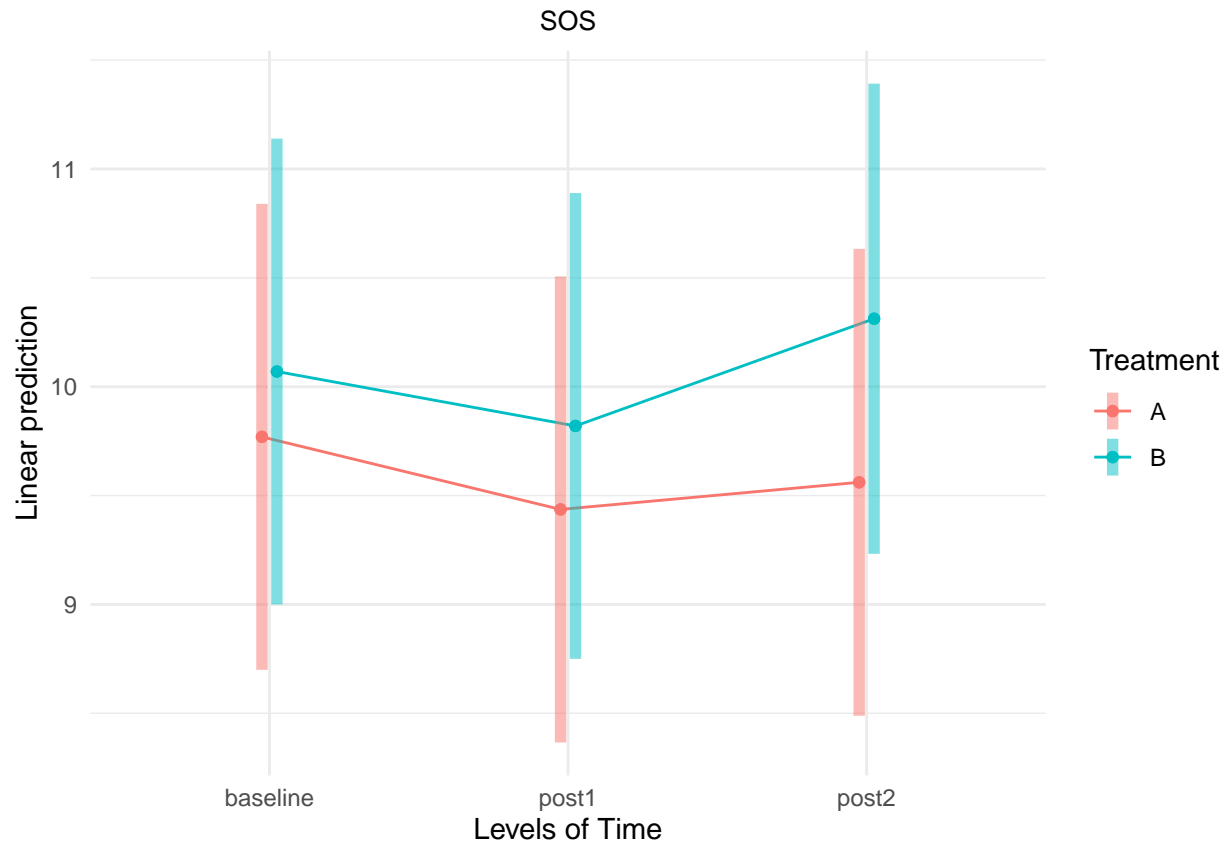
Example Interpretation: For OGA BWD A baseline - B baseline: the estimated difference between means is 1.09. Because the difference is positive this indicates that A baseline had a higher mean. (This can be confirmed using the graph or summary statistics). The corresponding p-value is 0.006. Recall that small p-values (typically less than 0.05) provide evidence of a difference.

2.2.5 Discussion

From the mixed model results, evidence of a difference for a treatment effect was observed. Further pairwise comparisons showed that for the baseline time point, there was evidence of a difference between the means of A and B. Time points post1 and post2 did not show evidence of a difference between the means of A and B. No evidence of a difference was found between any time points within treatments.

2.3 SOS

2.3.1 Graph



Example Interpretation: Estimated marginal means of each treatment across time points for SOS are displayed. The vertical lines represent the 95% confidence interval for the estimated marginal mean.

2.3.2 ANOVA Table

Table 4: Mixed Model Results

Response	Sum Sq	Mean Sq	NumDF	DenDF	F value	Raw Pr(>F)	Adj Pr(>F)
SOS.Time	4.2541	2.1270	2	168.1704	1.6196	0.2011	0.4739
SOS.Treatment	11.4830	11.4830	1	168.9162	8.7434	0.0036	0.0195
SOS.Time:Treatment	1.9496	0.9748	2	168.1729	0.7422	0.4776	0.7164

2.3.3 Pairwise Comparisons by Treatment

Table 5: Pairwise Comparisons by Treatment

contrast	Treatment	estimate	SE	df	t.ratio	p.value
baseline - post1	A	0.3333	0.2701	168.0044	1.2340	0.4348
baseline - post2	A	0.2088	0.2724	168.0304	0.7664	0.7241
post1 - post2	A	-0.1246	0.2724	168.0304	-0.4573	0.8912
baseline - post1	B	0.2500	0.2701	168.0044	0.9255	0.6249
baseline - post2	B	-0.2425	0.2811	168.3493	-0.8627	0.6645
post1 - post2	B	-0.4925	0.2811	168.3493	-1.7521	0.1891

Example Interpretation: For SOS A baseline - A post1: the estimated difference between means is 0.3333. Because the difference is positive this indicates that A baseline had a higher mean. (This can be confirmed using the graph or summary statistics). The corresponding Tukey-adjusted p-value is 0.4348. Recall that small p-values (typically less than 0.05) provide evidence of a difference.

2.3.4 Pairwise Comparisons by Time

Table 6: Pairwise Comparisons by Time

contrast	Time	estimate	SE	df	t.ratio	p.value
A - B	baseline	-0.3001	0.2732	168.4725	-1.0983	0.2736
A - B	post1	-0.3834	0.2732	168.4725	-1.4033	0.1624
A - B	post2	-0.7513	0.2829	168.2313	-2.6563	0.0087

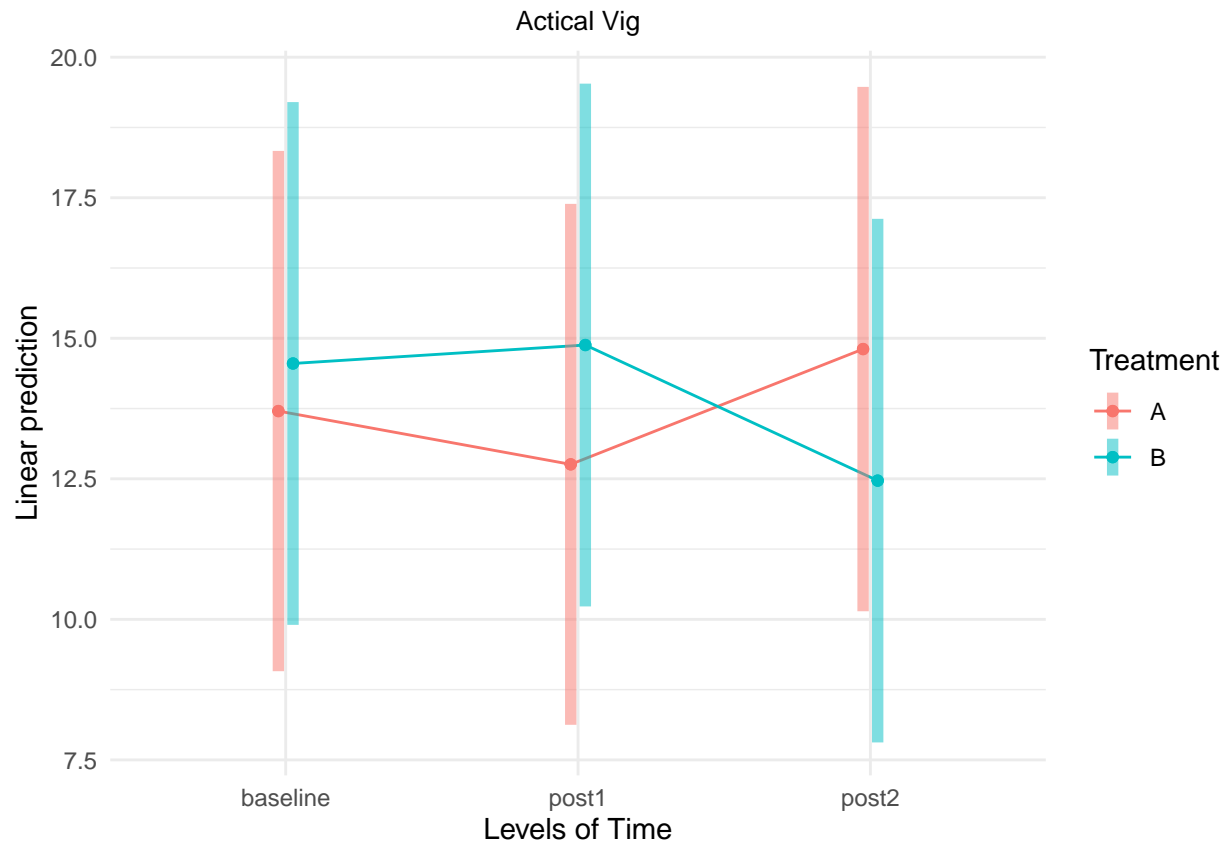
Example Interpretation: For SOS A baseline - B baseline : the estimated difference between means is -0.3. Because the difference is negative this indicates that A baseline had a lower mean. (This can be confirmed using the graph or summary statistics). The corresponding p-value is 0.2737. Recall that small p-values (typically less than 0.05) provide evidence of a difference.

2.3.5 Discussion

From the mixed model results, evidence of a difference for a treatment effect was observed. Further pairwise comparisons showed that for the post2 time point, there was evidence of a difference between the means of A and B. Time points baseline and post1 did not show evidence of a difference between the means of A and B. No evidence of a difference was found between any time points within treatments.

2.4 Actical Vig

2.4.1 Graph



Example Interpretation: Estimated marginal means of each treatment across time points for Actical are displayed. The vertical lines represent the 95% confidence interval for the estimated marginal mean.

2.4.2 ANOVA Table

Table 7: Mixed Model Results

Response	Sum Sq	Mean Sq	NumDF	DenDF	F value	Raw Pr(>F)	Adj Pr(>F)
VIG_MIN/DAY.Time	7.7867	3.8934	2	152.1055	0.2881	0.7501	0.8247
VIG_MIN/DAY.Treatment	1.9928	1.9928	1	152.5591	0.1475	0.7015	0.8247
VIG_MIN/DAY.Time:Treatment	160.4931	80.2466	2	152.1634	5.9379	0.0033	0.0195

2.4.3 Pairwise Comparisons by Treatment

Table 8: Pairwise Comparisons by Treatment

contrast	Treatment	estimate	SE	df	t.ratio	p.value
baseline - post1	A	0.9476	0.8747	153.0351	1.0834	0.5258
baseline - post2	A	-1.1028	0.9191	153.1340	-1.1999	0.4551
post1 - post2	A	-2.0505	0.9231	153.1049	-2.2212	0.0708
baseline - post1	B	-0.3281	0.9288	153.1089	-0.3532	0.9336
baseline - post2	B	2.0840	0.9448	153.2721	2.2058	0.0734
post1 - post2	B	2.4121	0.9414	153.2360	2.5622	0.0304

Example Interpretation: For Actical Vig A baseline - A post1: the estimated difference between means is 0.9476. Because the difference is positive this indicates that A baseline had a higher mean. (This can be confirmed using the graph or summary statistics). The corresponding Tukey-adjusted p-value is 0.5258. Recall that small p-values (typically less than 0.05) provide evidence of a difference.

2.4.4 Pairwise Comparisons by Time

Table 9: Pairwise Comparisons by Time

contrast	Time	estimate	SE	df	t.ratio	p.value
A - B	baseline	-0.8466	0.9100	153.2937	-0.9304	0.3536
A - B	post1	-2.1223	0.9191	153.3469	-2.3092	0.0223
A - B	post2	2.3403	0.9642	153.2998	2.4271	0.0164

Example Interpretation: For Actical Vig A baseline - B baseline : the estimated difference between means is -0.8466. Because the difference is negative this indicates that A baseline had a lower mean. (This can be confirmed using the graph or summary statistics). The corresponding p-value is 0.3536. Recall that small p-values (typically less than 0.05) provide evidence of a difference.

2.4.5 Discussion

From the mixed model results, evidence of a difference for a treatment*time effect was observed. Further pairwise comparisons showed that for the post1 time point, there was evidence of a difference between the means of A and B, with treatment B having a higher mean than treatment A. For the post2 time point, there was evidence of a difference between the means of A and B, with treatment A having a higher mean than treatment B. Time point baseline did not show evidence of a difference between the means of A and B. Evidence of a difference was found between post1 and post2 time points for treatment B.

3 Notes

- Patients 6 and 39 only have data for visits 1 and 2 (both pre-treatment). These dogs were dropped from the analysis, but should be mentioned in the paper.

- In the Actical data, patient 32 appeared to have a few data entry typos for the B1 block. Due to the arrangement of the rest of the patients, where B1, T1_1, T1_2, etc. appeared in order, these entries were changed manually in the data sheet to be B1 instead of B2 in a few cases and B3 in one case.
- We note that patient 31 had a small SED_MIN/DAY value (37.83) during the T2_1 phase compared to the other SED_MIN/Day values. This outlier was kept in the analysis.

4 Appendix

```
# Load in libraries
library(tidyverse)
library(readxl)
library(lme4)
library(emmeans)
library(openxlsx)
library(ggpubr)
library(performance)
library(stringr)

## Data Processing

# read in data
raw_data_sh1 = read_excel("Study Stats Platinum_Final_2-2-24_KM_3-6-24.xlsx")

raw_data_sh2 = read_excel("Study Stats Platinum_Final_2-2-24_KM_3-6-24.xlsx", sheet = "ACTIVITY")

# Set response variables of interest
response_variables = c("OGA.BWD_AL", "SOS", "CSOM.ACT(MEAN)",
                      "CSOM.BEHAV(MEAN)", "CBPI.PSS",
                      "CBPI.PIS", "CBPI.QOL", "SED_MIN/DAY", "LIGHT_MIN/DAY",
                      "VIG_MIN/DAY", "Total Daily Activity Count")

## Get treatment mapping
map_treatment = function(patient) {
  t1 = raw_data_sh2 %>%
    filter(`Enroll #` == patient,
           `B1 - T1 - B2 - T2` == "T1_1")
  t2 = raw_data_sh2 %>%
    filter(`Enroll #` == patient,
           `B1 - T1 - B2 - T2` == "T2_1")

  return(c(rep(t1[,]$`Supplement Administered`, 3),
           rep(t2[,]$`Supplement Administered`, 3)))
}

treatment = unlist(map(unique(raw_data_sh2$`Enroll #`), map_treatment))

# Code visits in terms of baseline, post1, post2
## Visits 2 and 5 will be coded as baseline
## Visits 3 and 6 will be coded as post1
## Visits 4 and 7 will be coded as post2
```



```

## Visit 1 will be coded as intro and dropped for purposes of analysis

## Just need analysis on OG_AL
clean_data_sh1 = raw_data_sh1 %>%
  rename(Patient = `Enroll #`) %>%
  mutate(Time = ifelse(Visit == 2 | Visit == 5, "baseline",
    ifelse(Visit == 3 | Visit == 6, "post1",
    ifelse(Visit == 4 | Visit == 7, "post2", "intro")))) %>%
  filter(Time != "intro") %>% # Drop visit 1 as not used in analysis
  select(-c(`Affected Limb (AL)`, Group, Visit, TX)) %>%
  cbind(Treatment = treatment) %>%
  filter(Patient != 39 & Patient != 6) %>% #Drop dogs 39 and 6, not needed in the analysis
  pivot_longer(!c(Treatment, Patient, Time), names_to = "Response", values_to = "Value")

clean_data_sh2 = raw_data_sh2 %>%
  rename(Time = `B1 - T1 - B2 - T2`, Patient = `Enroll #`) %>%
  mutate(Time = factor(Time, levels = c("B1", "T1_1", "T1_2",
    "B2", "T2_1", "T2_2"))) %>%

  group_by(Patient, Time) %>%
  summarise("SED_MIN/DAY" = mean(`SED_MIN/DAY`, na.rm = T),
    "LIGHT_MIN/DAY" = mean(`LIGHT_MIN/DAY`, na.rm = T),
    "VIG_MIN/DAY" = mean(`VIG_MIN/DAY`, na.rm = T),
    "Total Daily Activity Count" = mean(`Total Daily Activity Count`, na.rm = T)) %>%
  cbind(Treatment = treatment) %>%
  filter(Patient != 39 & Patient != 6) %>%
  mutate(Time = ifelse(Time == "B1" | Time == "B2", "baseline",
    ifelse(Time == "T1_1" | Time == "T2_1", "post1",
    ifelse(Time == "T1_2" | Time == "T2_2", "post2", "intro")))) %>%
  pivot_longer(!c(Treatment, Patient, Time), names_to = "Response", values_to = "Value")

# Combine data
clean_data = rbind(clean_data_sh1, clean_data_sh2) %>%
  filter(Response %in% response_variables)

## Summary Statistics
summary = clean_data %>%
  group_by(Response, Time, Treatment) %>%
  summarise(Min=min(Value, na.rm = T),
    Q1=quantile(Value, probs = 0.25, na.rm = T),
    Median=median(Value, na.rm = T),
    Mean = mean(Value, na.rm = T),
    Q3=quantile(Value, probs = 0.75, na.rm = T),
    Max=max(Value, na.rm = T),
    SD = sd(Value, na.rm = T),
    N = sum(!is.na(Value))) %>%
  arrange(Response, Treatment)

## Summary plots
create_boxplots = function(input) {
  data = clean_data %>%
    filter(Response == input)

```

```

p = ggplot(data, aes(x = Time, y = Value, color= Treatment)) +
  geom_boxplot() +
  ylab(input) +
  theme_minimal()
return(p)
}

boxplot_list = lapply(response_variables, create_boxplots)
names(boxplot_list) = response_variables

# Save all plots in pdf (one plot per page)
all_plots = ggexport(boxplot_list, filename = "boxplots.pdf")

## Analysis
fit_model = function(input) {
  data = clean_data %>%
    filter(Response == input)
  fit = lmerTest::lmer(Value ~ Time*Treatment + (1|Patient), data = data)
  return(fit)
}

model_list = map(response_variables, fit_model)
names(model_list) = response_variables

model_summary = lapply(model_list, stats::anova, type = 3)
names(model_summary) = response_variables

# Get a list of p-values from model summary
plist = as.data.frame(t(unlist(model_summary))) %>%
  select(matches(".Pr"))

# Find FDR adjusted p-values
plist_adjusted = p.adjust(plist, method = "fdr")

anova_df = as.data.frame(do.call(rbind, model_summary)) %>%
  cbind(plist_adjusted) %>%
  rownames_to_column() %>%
  rename(Response = rowname, "Adj Pr(>F)" = plist_adjusted, "Raw Pr(>F)" = `Pr(>F)`)

# Get pairwise comparisons for models with evidence of a difference
emmeans_vars = as.vector(filter(anova_df,
                                anova_df$`Adj Pr(>F)` <= 0.05)$Response)

emmeans_vars = emmeans_vars[stringr::str_detect(emmeans_vars, "Treatment")]
emmeans_vars = emmeans_vars %>%
  str_remove(".Time:Treatment") %>%
  str_remove(".Treatment")

emmeans_list1 = map(model_list[names(model_list) %in% emmeans_vars],
  emmeans, ~Time|Treatment)
emmeans_list2 = map(model_list[names(model_list) %in% emmeans_vars],
  emmeans, ~Treatment|Time)

```

```

pair_list1 = map(emmeans_list1, contrast, "pairwise")
pair_list2 = map(emmeans_list2, contrast, "pairwise")

# Create a df for contrasts
contrast_df1 = matrix(ncol = 8)
colnames(contrast_df1) = c("contrast", "Treatment", "estimate",
                           "SE", "df", "t.ratio",
                           "p.value", "Response")
for (i in 1:length(pair_list1)) {
  rows = summary(pair_list1[[i]])
  rows = cbind(rows, Response = rep(names(pair_list1)[[i]], 3))
  contrast_df1 = rbind(contrast_df1, rows)
}
contrast_df1 = contrast_df1 %>%
  relocate("Response")

# Create a df for contrasts
contrast_df2 = matrix(ncol = 8)
colnames(contrast_df2) = c("contrast", "Time", "estimate",
                           "SE", "df", "t.ratio",
                           "p.value", "Response")
for (i in 1:length(pair_list2)) {
  rows = summary(pair_list2[[i]])
  rows = cbind(rows, Response = rep(names(pair_list2)[[i]], 3))
  contrast_df2 = rbind(contrast_df2, rows)
}
contrast_df2 = contrast_df2 %>%
  relocate("Response")

# Create sheets for result excel file
list_of_datasets <- list("Summary Statistics" = summary,
                        "Mixed Model Results" = anova_df,
                        "Pairwise Comparisons|Treatment" = contrast_df1[-1,],
                        "Pairwise Comparisons|Time" = contrast_df2[-1,])
write.xlsx(list_of_datasets, file = "results.xlsx")

## Checking assumptions
resid_plots = map(model_list, plot)

# Checking log-transformed total daily activity count
total_act_data = clean_data %>%
  filter(Response == "Total Daily Activity Count") %>%
  mutate(Value = log(Value))

fit = lmerTest::lmer(Value ~ Time*Treatment + (1|Patient), data = total_act_data)
anova(fit)
plot(fit)

```