Calorimetry Plots

Abrar Al-Shaer

September 18, 2019

R Markdown

```
rm(list=ls())
library(ggplot2)
```

```
## Warning: package 'ggplot2' was built under R version 3.5.3
```

```
library(gplots)
```

```
## Warning: package 'gplots' was built under R version 3.5.2
```

```
##
## Attaching package: 'gplots'
```

```
## The following object is masked from 'package:stats':
##
##     lowess
```

```
library("dplyr")
```

```
## Warning: package 'dplyr' was built under R version 3.5.3
```

```
##
## Attaching package: 'dplyr'
```

```
## The following objects are masked from 'package:stats':
##
##     filter, lag
```

```
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```
library("ggpubr")
```

```
## Loading required package: magrittr
```

```
library(tidyverse)
```

```
## -- Attaching packages --------------------------------- tidyverse 1.2.1 --
```

```
## v tibble  2.1.3     v purrr   0.3.2
## v tidyr   0.8.3     v stringr 1.4.0
## v readr   1.3.1     v forcats 0.4.0
```

```
## Warning: package 'tibble' was built under R version 3.5.3
```

```
## Warning: package 'tidyr' was built under R version 3.5.3
```

```
## Warning: package 'readr' was built under R version 3.5.2
```

```
## Warning: package 'purrr' was built under R version 3.5.3
```

```
## Warning: package 'stringr' was built under R version 3.5.2
```

```
## Warning: package 'forcats' was built under R version 3.5.3
```

```
## -- Conflicts ------------------------------------ tidyverse_conflicts() --
## x tidyr::extract()   masks magrittr::extract()
## x dplyr::filter()    masks stats::filter()
## x dplyr::lag()       masks stats::lag()
## x purrr::set_names() masks magrittr::set_names()
```

```
library(car)
```

```
## Warning: package 'car' was built under R version 3.5.3
```

```
## Loading required package: carData
```

```
##
## Attaching package: 'car'
```

```
## The following object is masked from 'package:purrr':
##
##     some
```

```
## The following object is masked from 'package:dplyr':
##
##     recode
```

Reading in calorimetry dataset with outliers removed

```
calData <- read.csv("Calorimtery_ALX-FPR2_Calculated_Analysis_ALL_Mice_12Weeks-Old _RMoutliers_1
1.26.19.csv", header = TRUE, fill = TRUE, row.names = 1)
head(calData)
```

```
##     Genotype Diet Groups      RMR Resting.Gox Resting.Lox Total.VO2
## 122       KO  Con KO_Con 299.4064    8007.155   1542.3416  4434.463
## 123       KO  Con KO_Con 305.4159    7361.891   1189.3590  4382.593
## 191       KO  Con KO_Con 267.6917    7333.698   1463.1845  3992.102
## 195       KO  Con KO_Con 264.6629    6519.956   1097.3473  3621.593
## 398       KO  Con KO_Con 321.5926    7004.594    895.0995  4626.466
## 399       KO  Con KO_Con 349.0248    8036.956   1175.5389  4903.923
##     Total.VCO2 Total.EE Total.Gox Total.Lox  Total.XT VO2.Night  VO2.Day
## 122   3544.456 356.5519  8807.706  1488.834  552.9825  4755.168 3945.013
## 123   3640.134 355.0133  8255.969  1239.907  526.0125  4660.138 3940.616
## 191   3322.241 323.4836  7499.670  1118.668  288.2750  4115.417 3525.432
## 195   2948.360 292.1742  7014.011  1124.298  458.6750       NaN 3245.581
## 398   4109.049 379.7294  7860.372   864.086  741.0746  4821.000 4322.642
## 399   4439.167 403.9906  8063.124   776.143 1441.3534  5250.313 4403.623
##     VCO2.Night VCO2.Day EE.Night   EE.Day Gox.Night  Gox.Day Lox.Night
## 122   3620.203 3130.064 379.0361 316.7670 10024.392 7909.957 1897.9699
## 123   3692.920 3222.403 374.2232 318.3346  9349.356 7585.890 1615.2545
## 191   3329.297 2823.031 331.7581 283.5229  8038.106 6978.786 1312.8212
## 195   2982.858 2548.445 302.5225 260.1239  7575.940 6586.885 1313.5780
## 398   4347.135 3733.481 396.6935 352.8468  7981.248 7683.547  791.3548
## 399   4655.970 3952.054 430.6364 362.2785  8943.263 7350.389  992.5541
##       Lox.Day  XT.Night   XT.Day
## 122 1363.1927  787.3375 244.3125
## 123 1199.4161  694.9188 256.8375
## 191 1173.0097  424.3687 114.7562
## 195 1164.2169  682.8375 211.1625
## 398  983.8991  918.3962 461.6667
## 399       NaN 2186.5660 369.2830
```

```
#check structure of the data to make sure that "groups" the mice belong to are factor variables
str(calData)
```

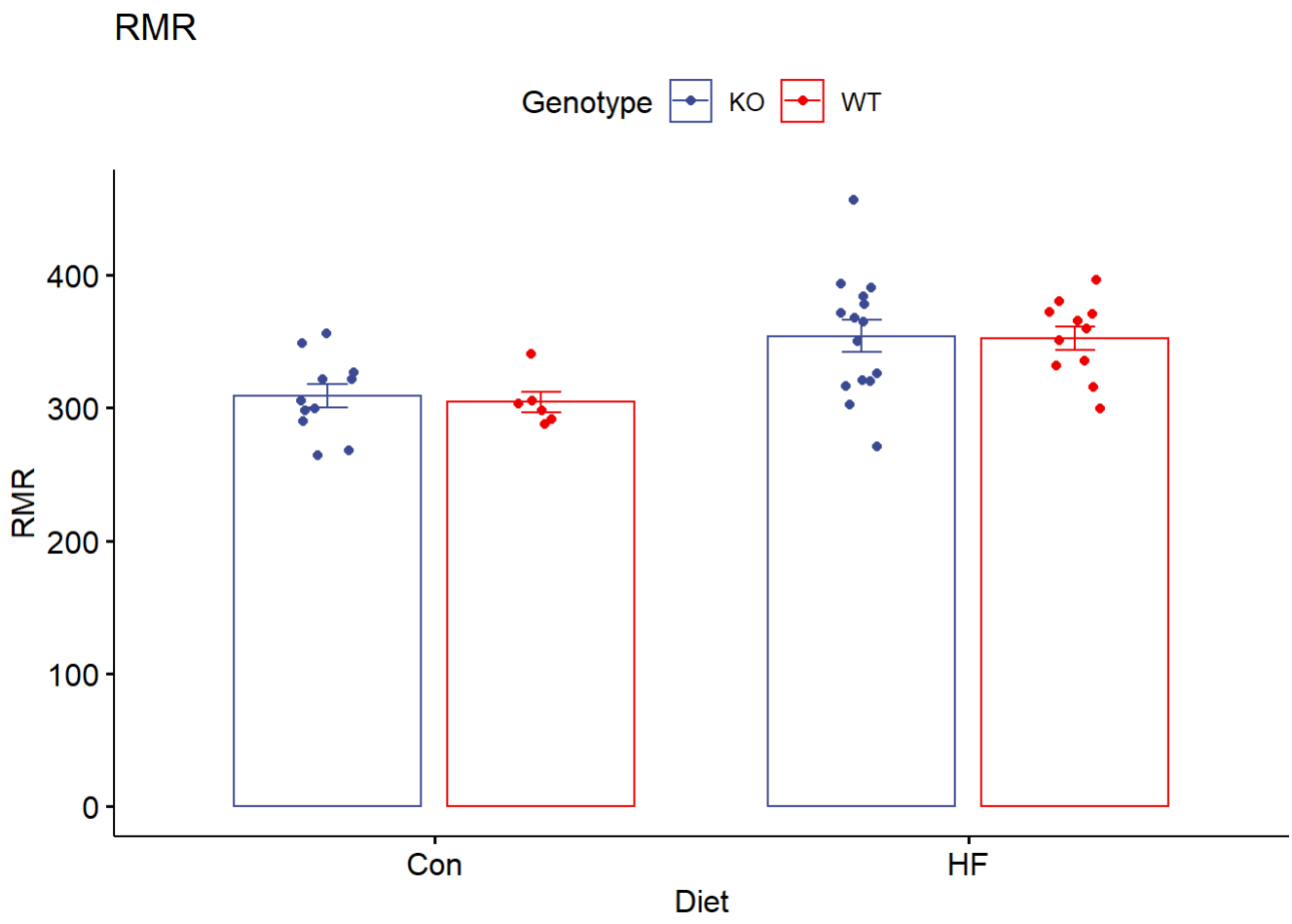
```
## 'data.frame':    44 obs. of  24 variables:
##  $ Genotype   : Factor w/ 2 levels "KO","WT": 1 1 1 1 1 1 1 1 1 1 ...
##  $ Diet       : Factor w/ 2 levels "Con","HF": 1 1 1 1 1 1 1 1 1 1 ...
##  $ Groups     : Factor w/ 4 levels "KO_Con","KO_HF",..: 1 1 1 1 1 1 1 1 1 1 ...
##  $ RMR        : num  299 305 268 265 322 ...
##  $ Resting.Gox: num  8007 7362 7334 6520 7005 ...
##  $ Resting.Lox: num  1542 1189 1463 1097 895 ...
##  $ Total.VO2  : num  4434 4383 3992 3622 4626 ...
##  $ Total.VCO2 : num  3544 3640 3322 2948 4109 ...
##  $ Total.EE   : num  357 355 323 292 380 ...
##  $ Total.Gox  : num  8808 8256 7500 7014 7860 ...
##  $ Total.Lox  : num  1489 1240 1119 1124 864 ...
##  $ Total.XT   : num  553 526 288 459 741 ...
##  $ VO2.Night  : num  4755 4660 4115 NaN 4821 ...
##  $ VO2.Day    : num  3945 3941 3525 3246 4323 ...
##  $ VCO2.Night : num  3620 3693 3329 2983 4347 ...
##  $ VCO2.Day   : num  3130 3222 2823 2548 3733 ...
##  $ EE.Night   : num  379 374 332 303 397 ...
##  $ EE.Day     : num  317 318 284 260 353 ...
##  $ Gox.Night  : num  10024 9349 8038 7576 7981 ...
##  $ Gox.Day    : num  7910 7586 6979 6587 7684 ...
##  $ Lox.Night  : num  1898 1615 1313 1314 791 ...
##  $ Lox.Day    : num  1363 1199 1173 1164 984 ...
##  $ XT.Night   : num  787 695 424 683 918 ...
##  $ XT.Day     : num  244 257 115 211 462 ...
```

Plotting Data & Statistical Analysis

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "RMR",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "RMR",
          position = position_dodge(0.8))
```

```
## Warning: Removed 1 rows containing non-finite values (stat_summary).
```

```
## Warning: Removed 1 rows containing missing values (geom_point).
```




```
#checking for normality
shapiro.test(calData$RMR)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$RMR
## W = 0.96598, p-value = 0.2283
```

```
#check for homogeneity of variance
leveneTest(RMR ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  1.8474 0.1546
##       39
```

```
#Run a 2-way ANOVA
#run the multiplicative model to see if there is an interaction term (signif. diff in slope)
#my_anova <- aov(RMR ~ Diet * Genotype, data = calData)
#Anova(my_anova, type = "III") #must run a type III ANOVA because the sample size is not balance
d

#run the additive model if the interaction term is not significant
#test if there is a significant difference in the intercepts of the lines
#if there is no significant interaction effect, then type II is more powerful. If interaction is
present, then type II is inappropriate while type III can still be used.
my_anova <- aov(RMR ~ Diet + Genotype, data = calData)
Anova(my_anova, type = "III")
```

```
## Anova Table (Type III tests)
##
## Response: RMR
##              Sum Sq Df   F value    Pr(>F)
## (Intercept) 1340677  1 1102.9494 < 2.2e-16 ***
## Diet          21799  1   17.9333 0.0001302 ***
## Genotype         81  1    0.0666 0.7977026
## Residuals     48622 40
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(my_anova)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = RMR ~ Diet + Genotype, data = calData)
##
## $Diet
##            diff      lwr      upr     p adj
## HF-Con 45.96723 23.98914 67.94532 0.0001334
##
## $Genotype
##            diff       lwr    upr     p adj
## WT-KO -2.799084 -24.77717 19.179 0.7981896
```

The above output shows that there is only a significant difference between the dietary groups, and not the genotypes.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "Resting.Gox",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "Resting Glucose Metabolism",
          position = position_dodge(0.8))
```

```
## Warning: Removed 2 rows containing non-finite values (stat_summary).
```

```
## Warning: Removed 2 rows containing missing values (geom_point).
```


```
#checking for normality
shapiro.test(calData$Resting.Gox)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$Resting.Gox
## W = 0.95009, p-value = 0.06504
```

```
#check for homogeneity of variance
leveneTest(Resting.Gox ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  1.0793 0.3694
##       38
```

```
#Run a 2-way ANOVA
#run the multiplicative model to see if there is an interaction term (signif. diff in slope)
my_anova <- aov(Resting.Gox ~ Diet + Genotype, data = calData)
Anova(my_anova, type = "III") #must run a type III ANOVA because the sample size is not balanced
```

```
## Anova Table (Type III tests)
##
## Response: Resting.Gox
##                Sum Sq Df   F value    Pr(>F)
## (Intercept) 789891258  1 1326.5446 < 2.2e-16 ***
## Diet         58860004  1   98.8496 3.014e-12 ***
## Genotype        83152  1    0.1396    0.7107
## Residuals    23222559 39
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(my_anova)
```

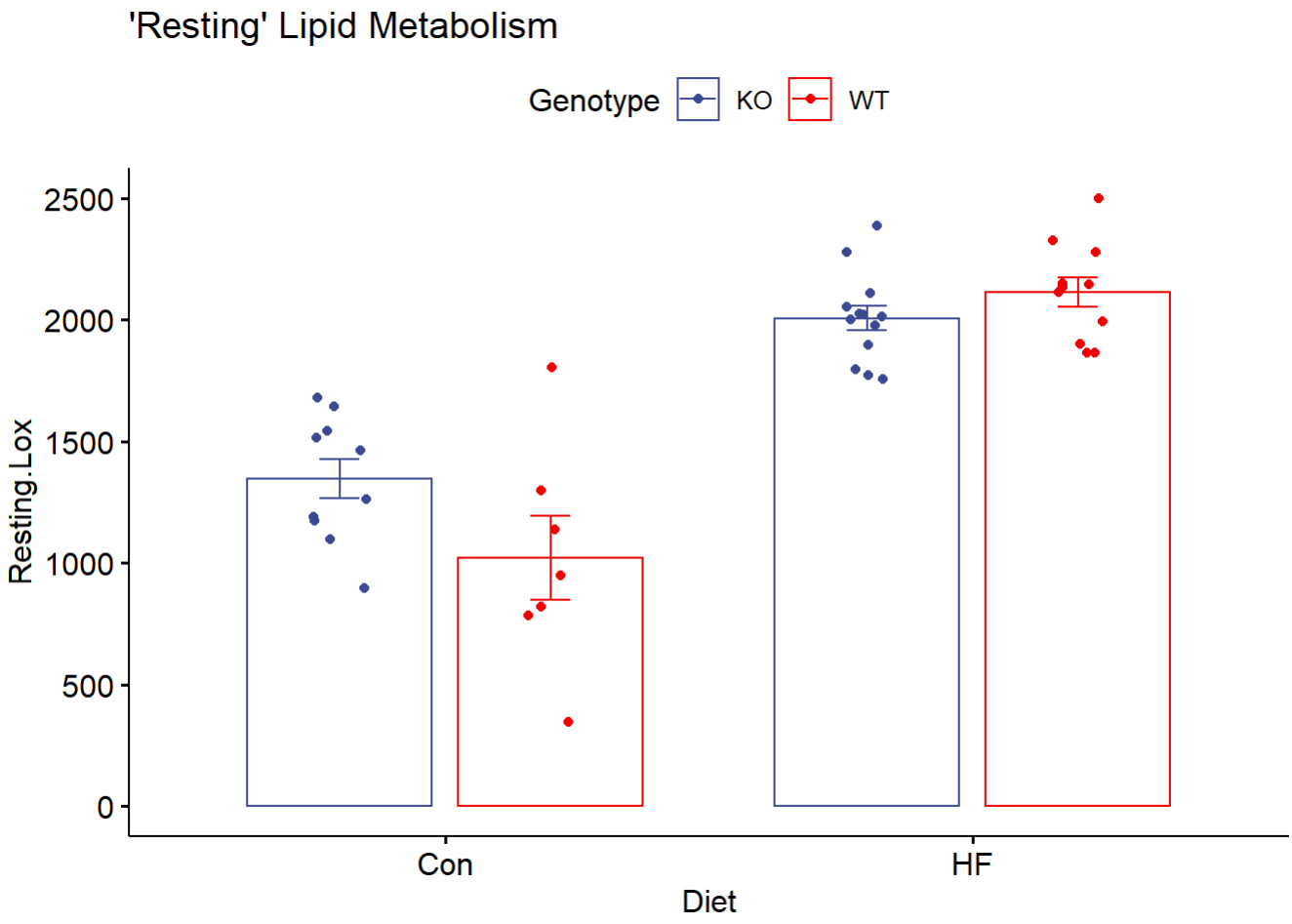
```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = Resting.Gox ~ Diet + Genotype, data = calData)
##
## $Diet
##            diff      lwr      upr p adj
## HF-Con 2390.594 1903.924 2877.265     0
##
## $Genotype
##            diff      lwr     upr     p adj
## WT-KO -90.61398 -581.276 400.048 0.7107662
```

The above output shows that there is only a significant difference between the dietary groups, and not the genotypes.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "Resting.Lox",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "'Resting' Lipid Metabolism",
          position = position_dodge(0.8))
```

```
## Warning: Removed 3 rows containing non-finite values (stat_summary).
```

```
## Warning: Removed 3 rows containing missing values (geom_point).
```




```
#checking for normality
shapiro.test(calData$Resting.Lox)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$Resting.Lox
## W = 0.93766, p-value = 0.02613
```

```
#check for homogeneity of variance
leveneTest(Resting.Lox ~ Groups, data = calData)
```

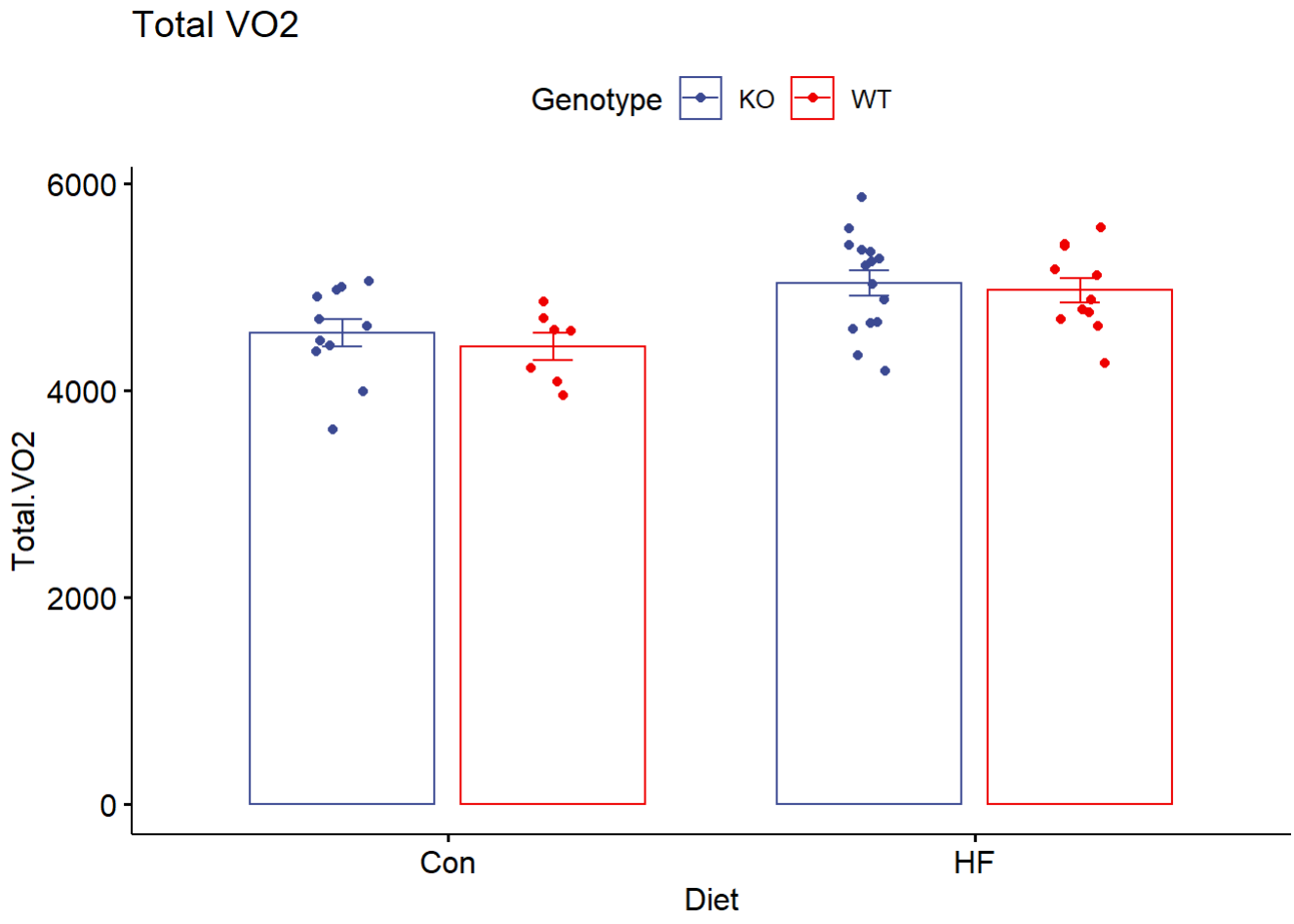
```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value  Pr(>F)
## group  3  2.5204 0.07285 .
##       37
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#run a wilcoxon test because data was not normal
compare_means(Resting.Lox ~ Groups, data = calData, method = "wilcox")
```

```
## # A tibble: 6 x 8
##   .y.         group1 group2         p   p.adj p.format p.signif method
##   <chr>       <chr>  <chr>      <dbl>   <dbl> <chr>    <chr>    <chr>
## 1 Resting.Lox KO_Con KO_HF  0.0000633 0.00038 6.3e-05  ****     Wilcoxon
## 2 Resting.Lox KO_Con WT_Con 0.107     0.21    0.10735  ns       Wilcoxon
## 3 Resting.Lox KO_Con WT_HF  0.000124  0.00062 0.00012  ***      Wilcoxon
## 4 Resting.Lox KO_HF  WT_Con 0.000874  0.0026  0.00087  ***      Wilcoxon
## 5 Resting.Lox KO_HF  WT_HF  0.183     0.21    0.18268  ns       Wilcoxon
## 6 Resting.Lox WT_Con WT_HF  0.000578  0.0023  0.00058  ***      Wilcoxon
```

The data above was not normal therefore we used a wilcoxon test. No signficant differences observed between the genotypes - only a modest increase in “resting” lipid metabolism with the KO mice compared to the WTs (p = 0.1)

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "Total.VO2",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "Total VO2",
          position = position_dodge(0.8))
```




```
#checking for normality
shapiro.test(calData$Total.VO2)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$Total.VO2
## W = 0.99179, p-value = 0.987
```

```
#check for homogeneity of variance
leveneTest(Total.VO2 ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  0.2698 0.8468
##       40
```

```
#Run a 2-way ANOVA
#run the multiplicative model to see if there is an interaction term (signif. diff in slope)
my_anova <- aov(Total.VO2 ~ Diet + Genotype, data = calData)
Anova(my_anova, type = "III") #must run a type III ANOVA because the sample size is not balanced
```

```
## Anova Table (Type III tests)
##
## Response: Total.VO2
##                Sum Sq Df   F value    Pr(>F)
## (Intercept) 295983493  1 1630.6810 < 2.2e-16 ***
## Diet          2742855  1   15.1114 0.0003632 ***
## Genotype       100718  1    0.5549 0.4605743
## Residuals     7441874 41
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(my_anova)
```

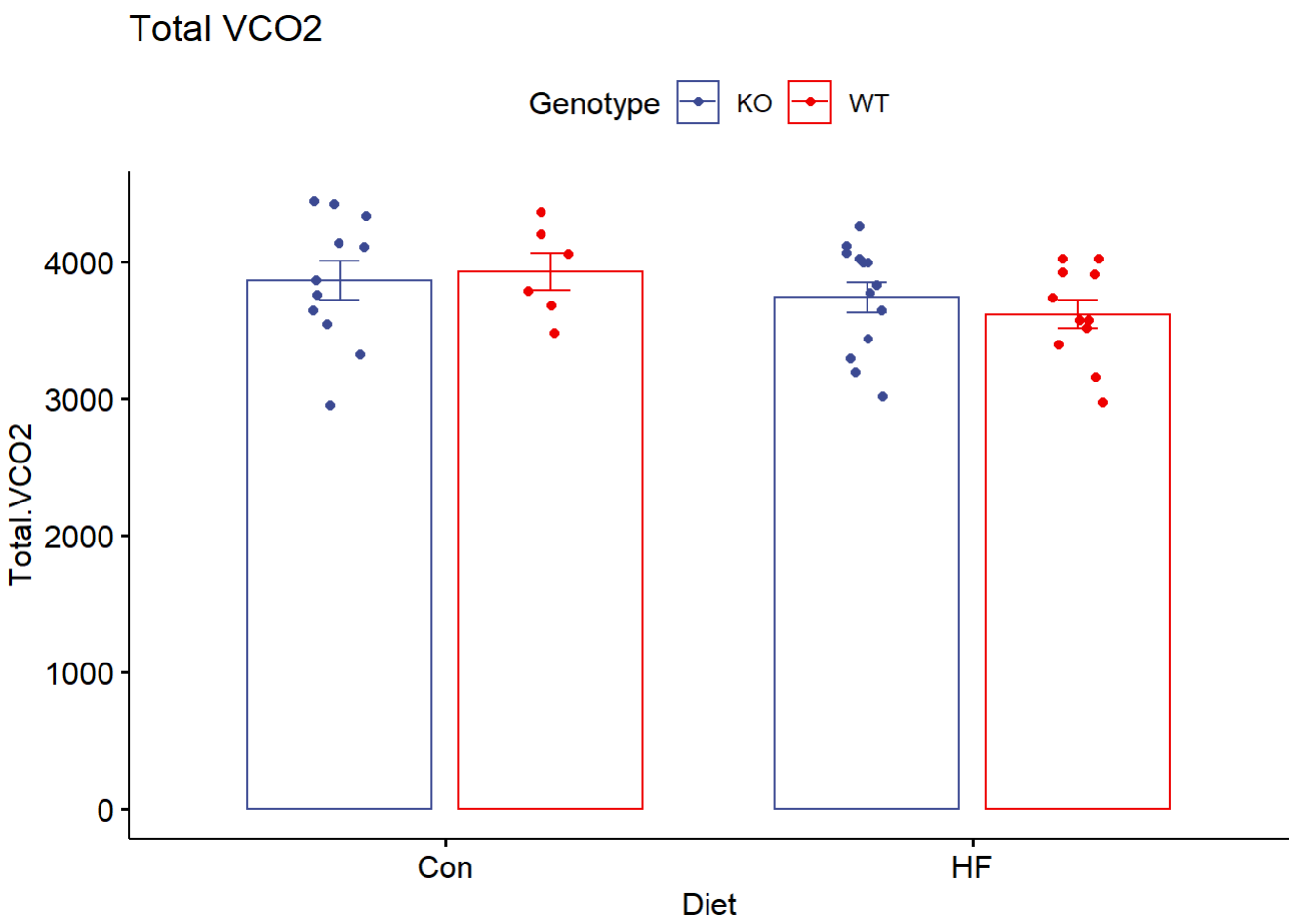
```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = Total.VO2 ~ Diet + Genotype, data = calData)
##
## $Diet
##            diff      lwr     upr     p adj
## HF-Con 504.7824 240.9639 768.601 0.0003894
##
## $Genotype
##            diff       lwr      upr     p adj
## WT-KO -97.25303 -361.0716 166.5655 0.4608348
```

No diet-genotype interactions. Only significant difference between diets.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "Total.VCO2",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "Total VCO2",
          position = position_dodge(0.8))
```

```
## Warning: Removed 3 rows containing non-finite values (stat_summary).
```

```
## Warning: Removed 3 rows containing missing values (geom_point).
```




```
#checking for normality
shapiro.test(calData$Total.VCO2)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$Total.VCO2
## W = 0.9707, p-value = 0.3634
```

```
#check for homogeneity of variance
leveneTest(Total.VCO2 ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3   0.534 0.6618
##       37
```

```
#Run a 2-way ANOVA
#run the multiplicative model to see if there is an interaction term (signif. diff in slope)
my_anova <- aov(Total.VCO2 ~ Diet + Genotype, data = calData)
Anova(my_anova, type = "III") #must run a type III ANOVA because the sample size is not balanced
```

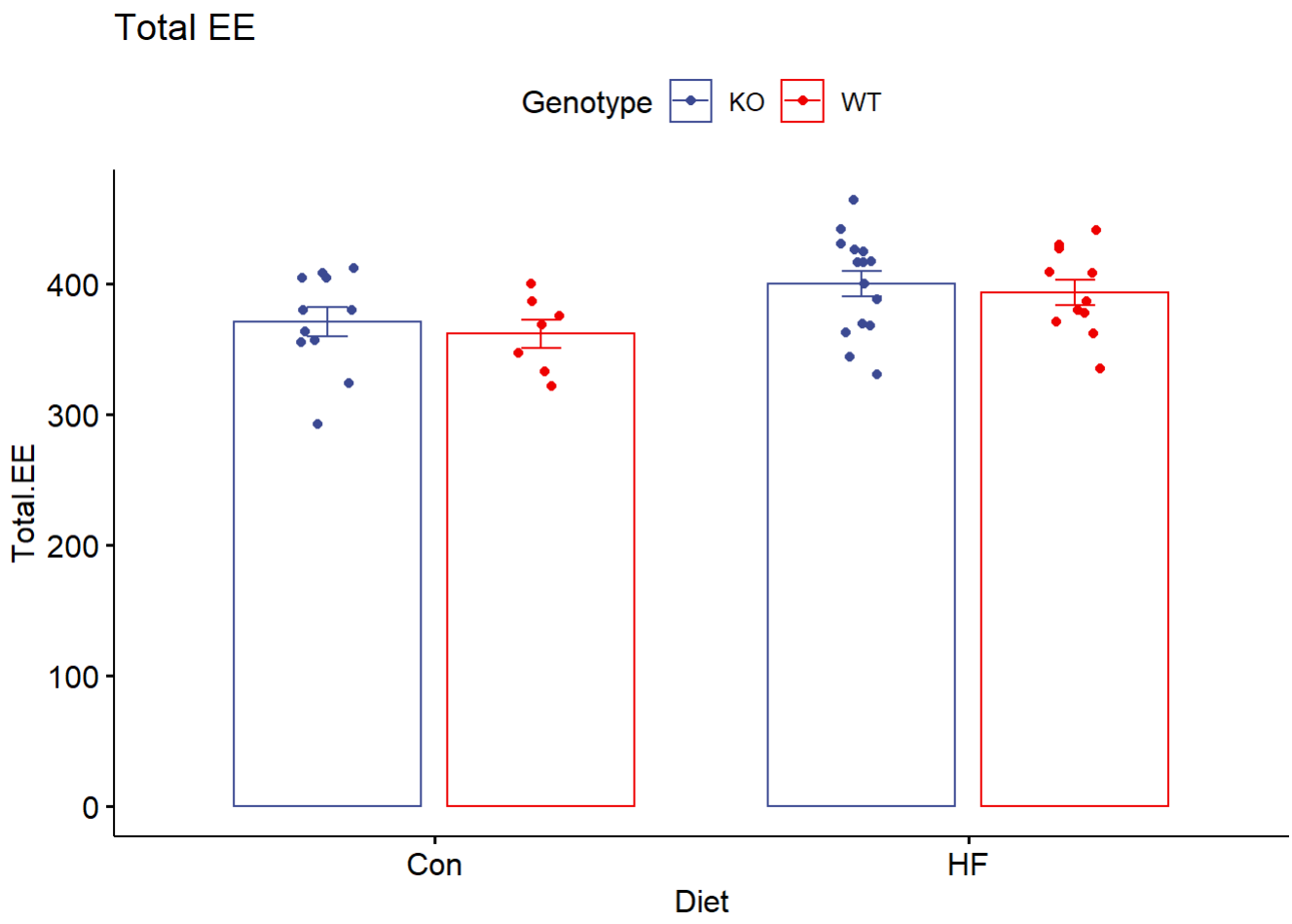
```
## Anova Table (Type III tests)
##
## Response: Total.VCO2
##                Sum Sq Df   F value Pr(>F)
## (Intercept) 213300770  1 1349.4026 <2e-16 ***
## Diet           388699  1    2.4590 0.1251
## Genotype        25486  1    0.1612 0.6903
## Residuals     6006680 38
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(my_anova)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = Total.VCO2 ~ Diet + Genotype, data = calData)
##
## $Diet
##             diff       lwr      upr    p adj
## HF-Con -204.1075 -459.2498 51.03471 0.113618
##
## $Genotype
##            diff       lwr      upr     p adj
## WT-KO -50.32586 -305.4681 204.8164 0.6919044
```

No diet-genotype interaction. Only significant difference between diets.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "Total.EE",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "Total EE",
          position = position_dodge(0.8))
```




```
#checking for normality
shapiro.test(calData$Total.EE)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$Total.EE
## W = 0.9867, p-value = 0.8857
```

```
#check for homogeneity of variance
leveneTest(Total.EE ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  0.1945 0.8995
##       40
```

```
#Run a 2-way ANOVA
#run the multiplicative model to see if there is an interaction term (signif. diff in slope)
my_anova <- aov(Total.EE ~ Diet + Genotype, data = calData)
Anova(my_anova, type = "III") #must run a type III ANOVA because the sample size is not balanced
```

```
## Anova Table (Type III tests)
##
## Response: Total.EE
##              Sum Sq Df   F value    Pr(>F)
## (Intercept) 1963406  1 1604.4310 < 2.2e-16 ***
## Diet           9601  1    7.8460  0.007737 **
## Genotype        633  1    0.5171  0.476179
## Residuals     50173 41
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(my_anova)
```

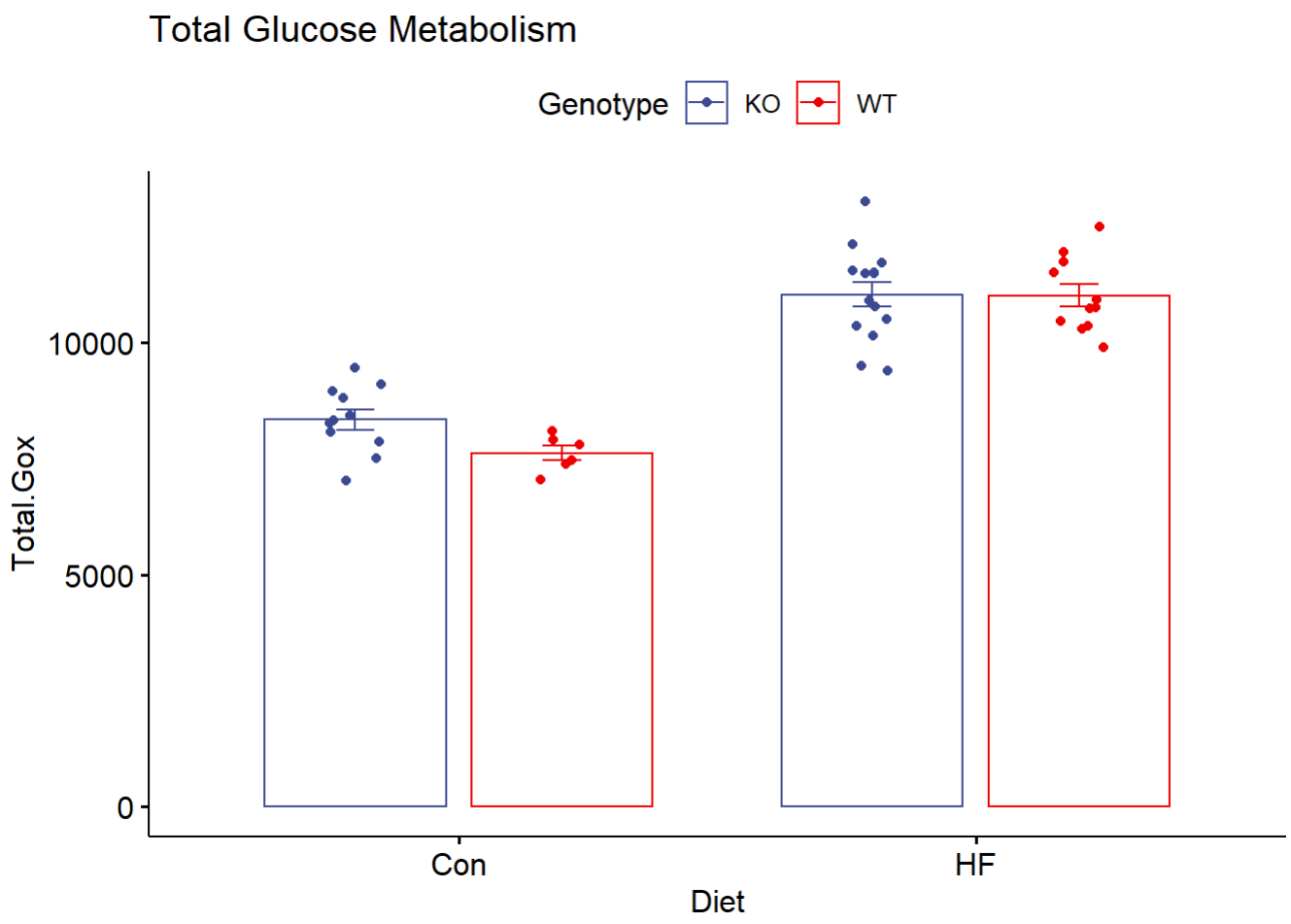
```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = Total.EE ~ Diet + Genotype, data = calData)
##
## $Diet
##            diff      lwr      upr     p adj
## HF-Con 29.79864 8.136539 51.46074 0.0082106
##
## $Genotype
##           diff       lwr      upr     p adj
## WT-KO -7.70837 -29.37047 13.95373 0.4764353
```

No diet-genotype interaction. Only significant difference between diets.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "Total.Gox",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "Total Glucose Metabolism",
          position = position_dodge(0.8))
```

```
## Warning: Removed 2 rows containing non-finite values (stat_summary).
```

```
## Warning: Removed 2 rows containing missing values (geom_point).
```




```
#checking for normality
shapiro.test(calData$Total.Gox)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$Total.Gox
## W = 0.95275, p-value = 0.08103
```

```
#check for homogeneity of variance
leveneTest(Total.Gox ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  1.4617 0.2403
##       38
```

```
#Run a 2-way ANOVA
#run the multiplicative model to see if there is an interaction term (signif. diff in slope)
my_anova <- aov(Total.Gox ~ Diet + Genotype, data = calData)
Anova(my_anova, type = "III") #must run a type III ANOVA because the sample size is not balanced
```

```
## Anova Table (Type III tests)
##
## Response: Total.Gox
##                Sum Sq Df   F value    Pr(>F)
## (Intercept) 941580275  1 1369.0716 < 2.2e-16 ***
## Diet         88180855  1  128.2163  6.76e-14 ***
## Genotype       866983  1    1.2606    0.2684
## Residuals    26822287 39
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(my_anova)
```

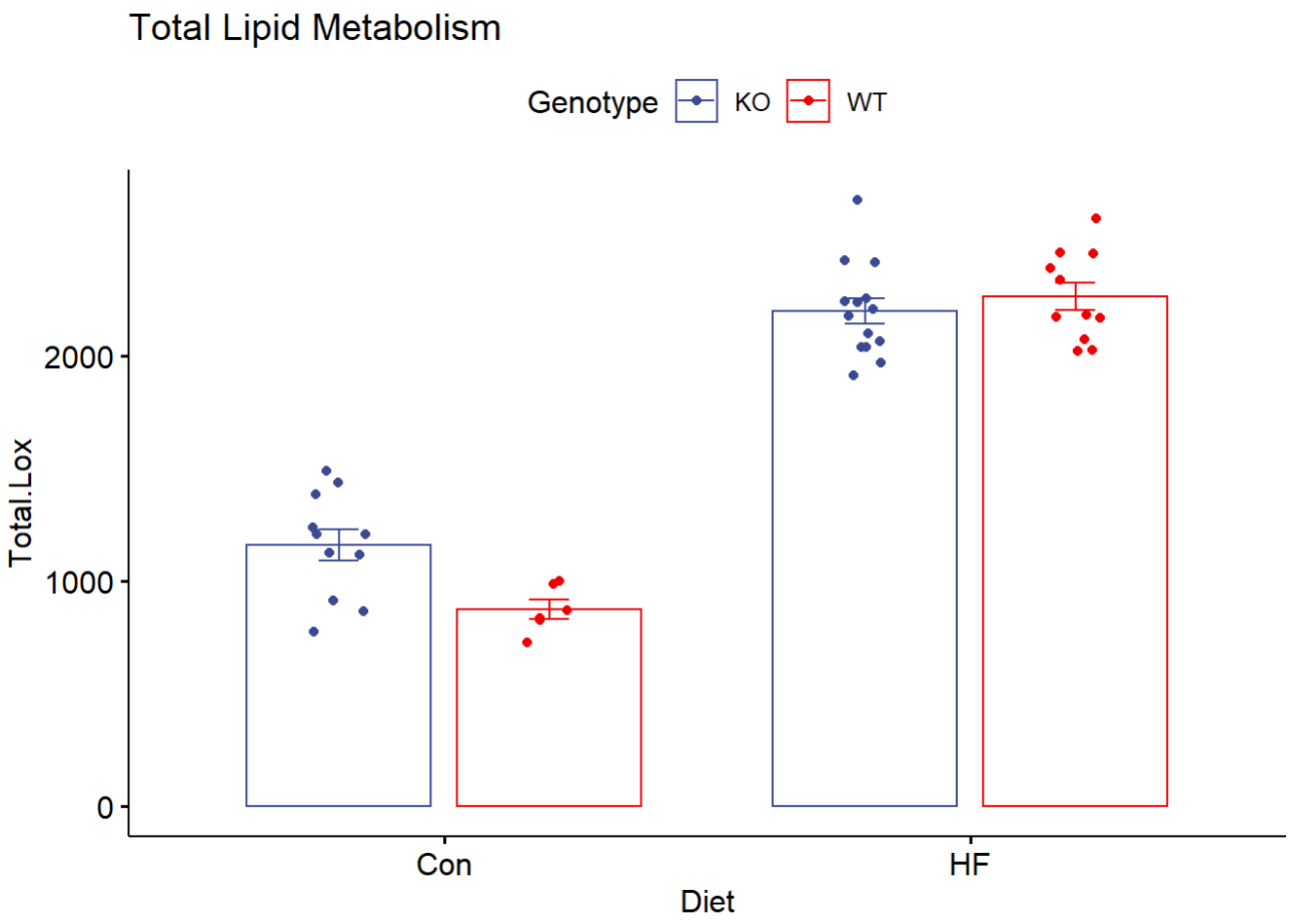
```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = Total.Gox ~ Diet + Genotype, data = calData)
##
## $Diet
##            diff      lwr      upr p adj
## HF-Con 2937.678 2410.356 3464.999     0
##
## $Genotype
##            diff       lwr      upr     p adj
## WT-KO -291.5972 -818.9185 235.7241 0.2701941
```

No diet-genotype interaction. Only significant difference between diets.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "Total.Lox",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "Total Lipid Metabolism",
          position = position_dodge(0.8))
```

```
## Warning: Removed 2 rows containing non-finite values (stat_summary).
```

```
## Warning: Removed 2 rows containing missing values (geom_point).
```




```
#checking for normality
shapiro.test(calData$Total.Lox)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$Total.Lox
## W = 0.88583, p-value = 0.0005569
```

```
#check for homogeneity of variance
leveneTest(Total.Lox ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  0.8271 0.4872
##       38
```

```
#run a wilcoxon test because data was not normal
compare_means(Total.Lox ~ Groups, data = calData, method = "wilcox")
```

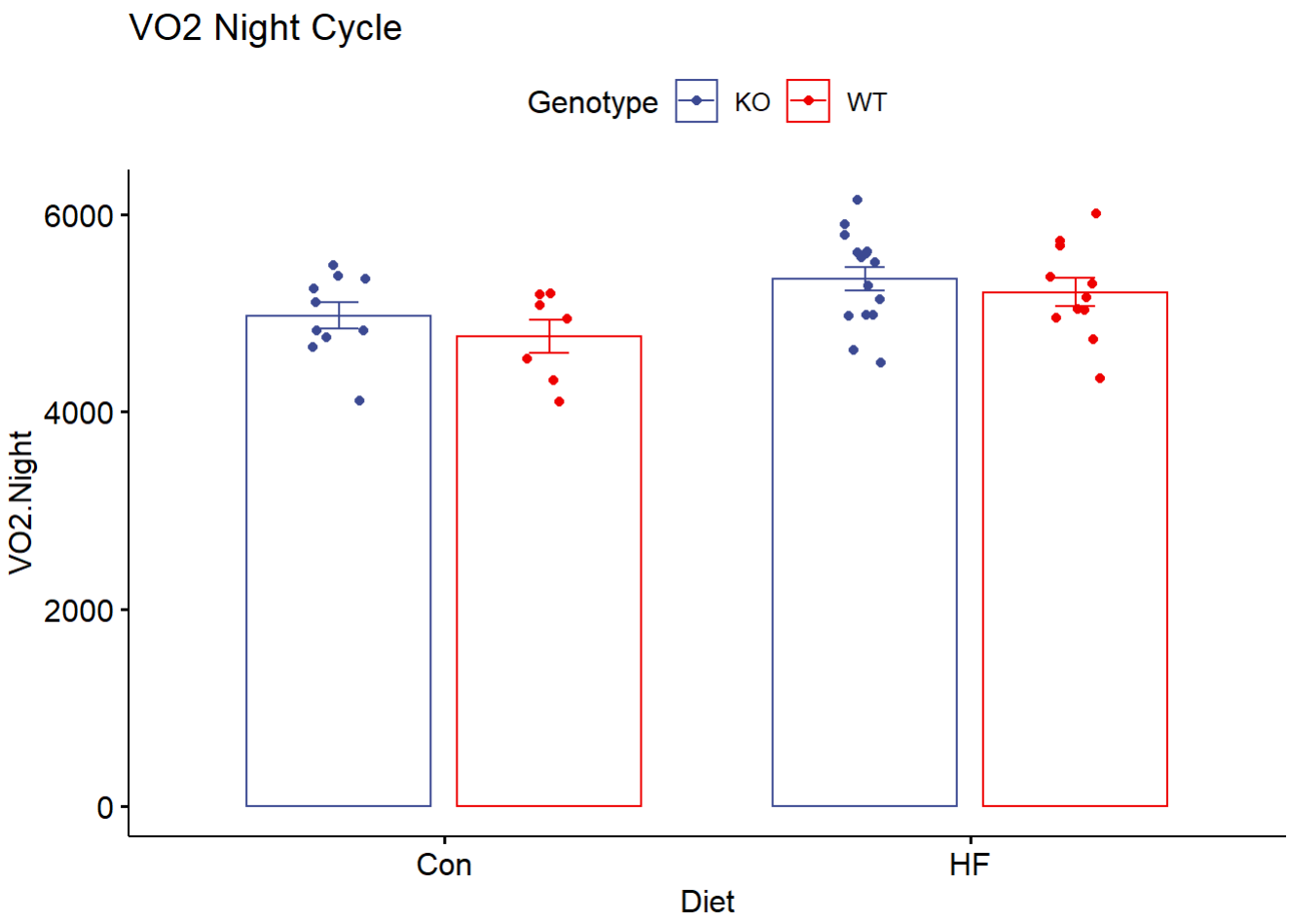
```
## # A tibble: 6 x 8
##   .y.       group1 group2         p   p.adj p.format p.signif method
##   <chr>     <chr>  <chr>      <dbl>   <dbl> <chr>    <chr>    <chr>
## 1 Total.Lox KO_Con KO_HF  0.0000281 0.00017 2.8e-05  ****     Wilcoxon
## 2 Total.Lox KO_Con WT_Con 0.0237    0.047   0.02374  *        Wilcoxon
## 3 Total.Lox KO_Con WT_HF  0.0000815 0.00041 8.2e-05  ****     Wilcoxon
## 4 Total.Lox KO_HF  WT_Con 0.000620  0.0025  0.00062  ***      Wilcoxon
## 5 Total.Lox KO_HF  WT_HF  0.529     0.53    0.52898  ns       Wilcoxon
## 6 Total.Lox WT_Con WT_HF  0.00109   0.0033  0.00109  **       Wilcoxon
```

The data above was not normal therefore we used a wilcoxon test. There is a signgicant difference between KO Con and WT Con. There is a significant increase in total lipid metabolism with the control KO mice compared to the WTs (p = 0.02). However this difference is lost upon a high fat diet. This indicates that at baseline the knockout of FPR2 causes the KO mice to have higher total lipid metabolism compared to the WT mice, however this effect is lost when the mice are challeged with a HF diet.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "VO2.Night",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "VO2 Night Cycle",
          position = position_dodge(0.8))
```

```
## Warning: Removed 1 rows containing non-finite values (stat_summary).
```

```
## Warning: Removed 1 rows containing missing values (geom_point).
```




```
#checking for normality
shapiro.test(calData$VO2.Night)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$VO2.Night
## W = 0.9886, p-value = 0.9415
```

```
#check for homogeneity of variance
leveneTest(VO2.Night ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  0.0518 0.9842
##       39
```

```
#Run a 2-way ANOVA
#run the multiplicative model to see if there is an interaction term (signif. diff in slope)
my_anova <- aov(VO2.Night ~ Diet + Genotype, data = calData)
Anova(my_anova, type = "III") #must run a type III ANOVA because the sample size is not balanced
```

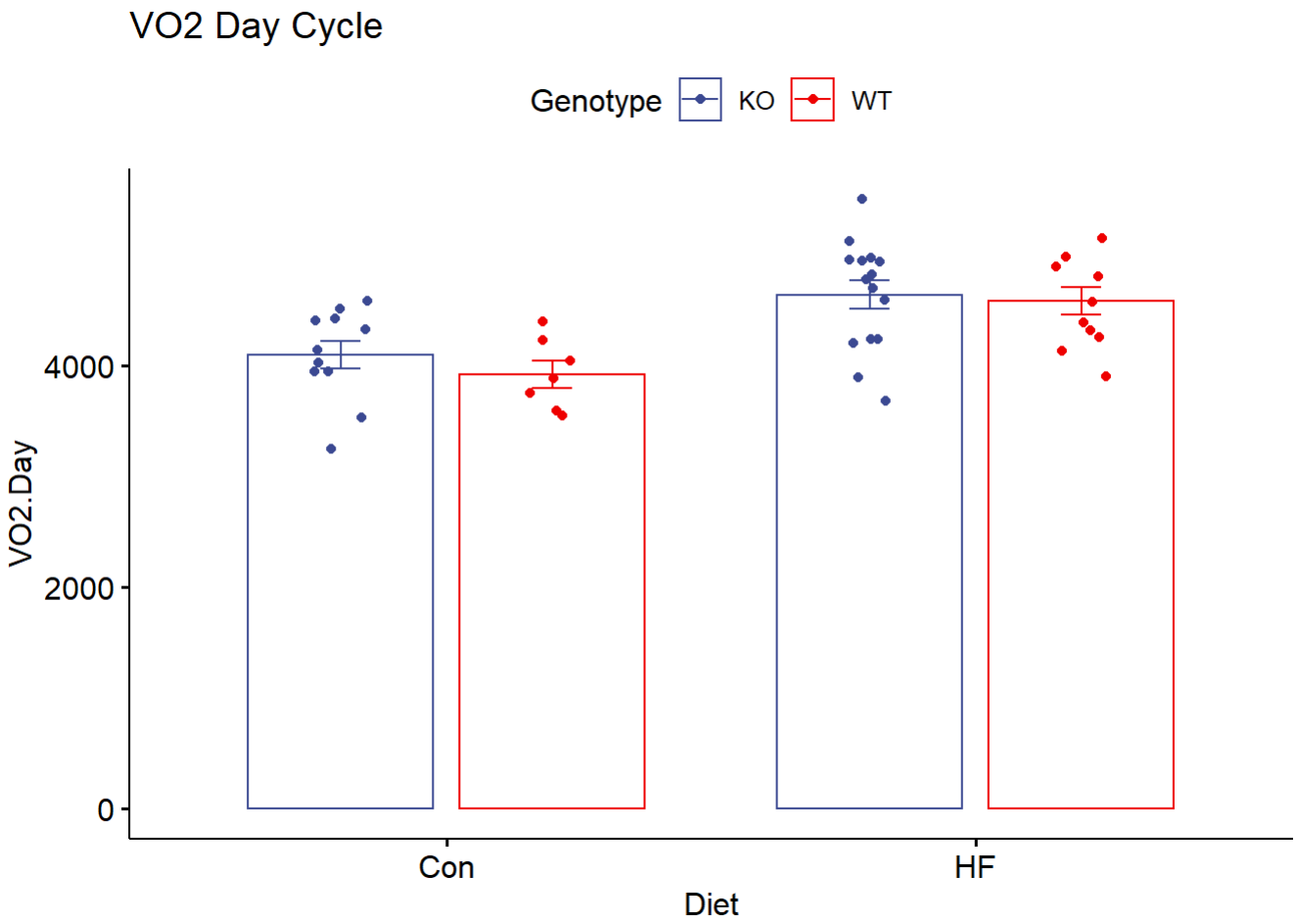
```
## Anova Table (Type III tests)
##
## Response: VO2.Night
##                Sum Sq Df   F value    Pr(>F)
## (Intercept) 327413332  1 1601.9490 < 2.2e-16 ***
## Diet          1688056  1    8.2592  0.006463 **
## Genotype       281237  1    1.3760  0.247718
## Residuals     8175375 40
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(my_anova)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = VO2.Night ~ Diet + Genotype, data = calData)
##
## $Diet
##            diff     lwr      upr     p adj
## HF-Con 403.4149 118.425 688.4048 0.0066856
##
## $Genotype
##           diff       lwr      upr     p adj
## WT-KO -163.922 -446.3673 118.5233 0.2477473
```

No diet-genotype interaction. Only significant difference between diets.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "VO2.Day",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "VO2 Day Cycle",
          position = position_dodge(0.8))
```




```
#checking for normality
shapiro.test(calData$VO2.Day)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$VO2.Day
## W = 0.98439, p-value = 0.8066
```

```
#check for homogeneity of variance
leveneTest(VO2.Day ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  0.3968  0.756
##       40
```

```
#Run a 2-way ANOVA
#run the multiplicative model to see if there is an interaction term (signif. diff in slope)
my_anova <- aov(VO2.Day ~ Diet + Genotype, data = calData)
Anova(my_anova, type = "III") #must run a type III ANOVA because the sample size is not balanced
```

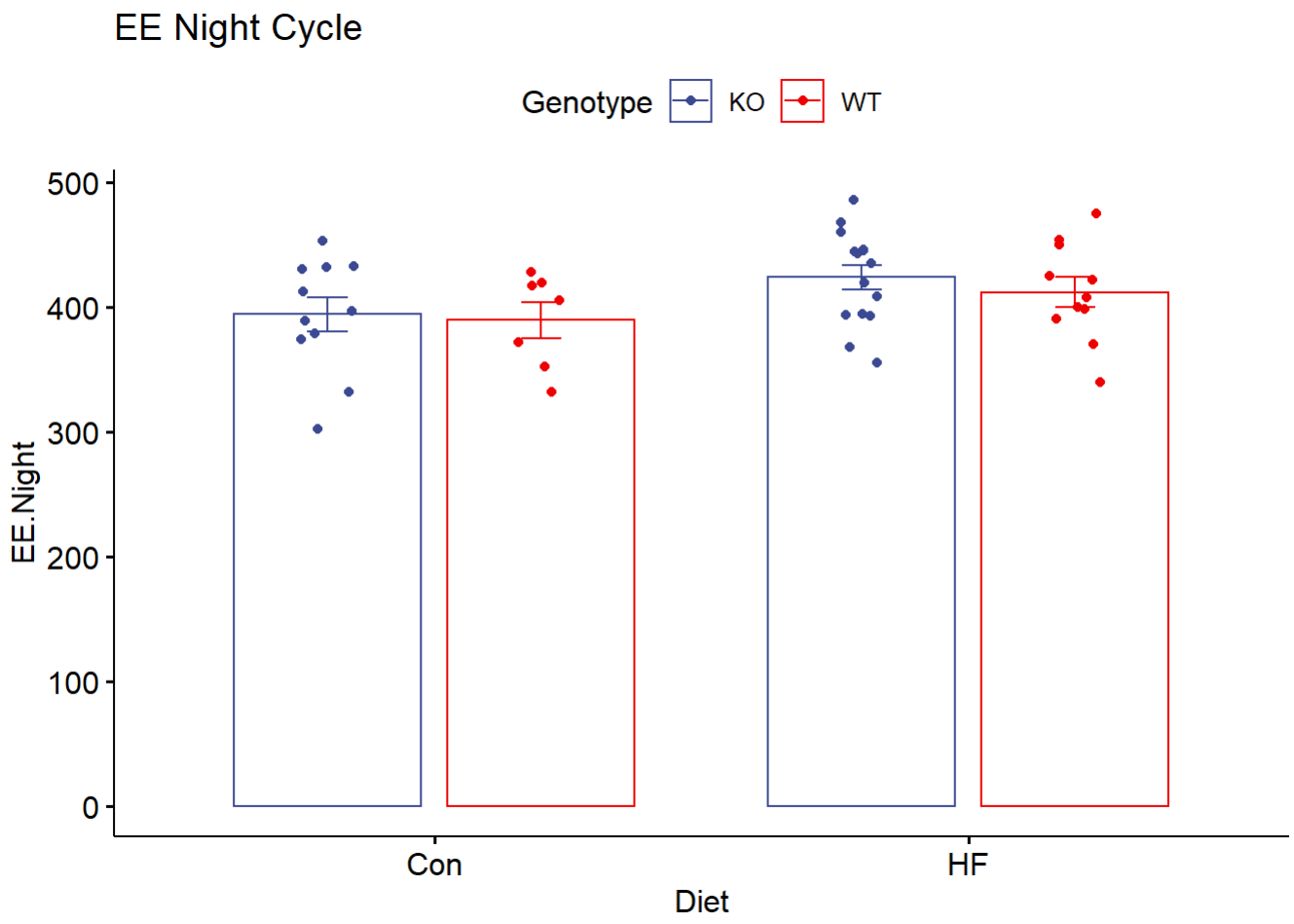
```
## Anova Table (Type III tests)
##
## Response: VO2.Day
##                Sum Sq Df   F value    Pr(>F)
## (Intercept) 237167650  1 1296.8551 < 2.2e-16 ***
## Diet          3673754  1   20.0884  5.83e-05 ***
## Genotype       119935  1    0.6558    0.4227
## Residuals     7498042 41
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(my_anova)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = VO2.Day ~ Diet + Genotype, data = calData)
##
## $Diet
##           diff      lwr      upr    p adj
## HF-Con 584.415 319.6028 849.2273 6.31e-05
##
## $Genotype
##           diff       lwr      upr    p adj
## WT-KO -106.126 -370.9383 158.6862 0.422986
```

No diet-genotype interaction. Only significant difference between diets.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "EE.Night",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "EE Night Cycle",
          position = position_dodge(0.8))
```




```
#checking for normality
shapiro.test(calData$EE.Night)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$EE.Night
## W = 0.98116, p-value = 0.6802
```

```
#check for homogeneity of variance
leveneTest(EE.Night ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  0.1061 0.9561
##       40
```

```
#Run a 2-way ANOVA
#run the multiplicative model to see if there is an interaction term (signif. diff in slope)
my_anova <- aov(EE.Night ~ Diet + Genotype, data = calData)
Anova(my_anova, type = "III") #must run a type III ANOVA because the sample size is not balanced
```

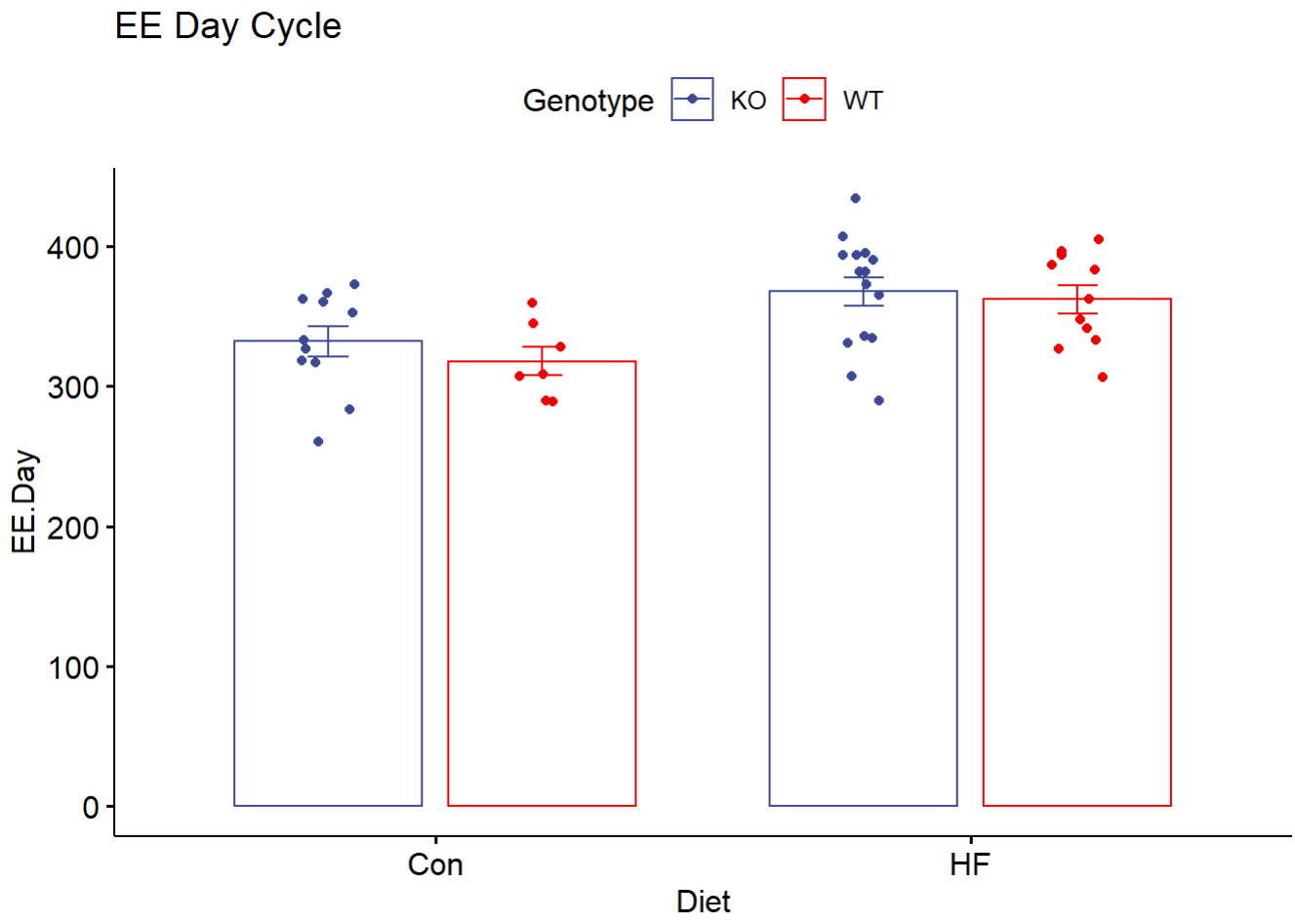
```
## Anova Table (Type III tests)
##
## Response: EE.Night
##              Sum Sq Df   F value  Pr(>F)
## (Intercept) 2243964  1 1426.8555 < 2e-16 ***
## Diet           7690  1    4.8897 0.03265 *
## Genotype        857  1    0.5449 0.46462
## Residuals     64479 41
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(my_anova)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = EE.Night ~ Diet + Genotype, data = calData)
##
## $Diet
##            diff      lwr     upr     p adj
## HF-Con 26.59697 2.040046 51.1539 0.0344782
##
## $Genotype
##            diff       lwr      upr     p adj
## WT-KO -8.970644 -33.52757 15.58628 0.4648752
```

No diet-genotype interaction. Only significant difference between diets.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "EE.Day",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "EE Day Cycle",
          position = position_dodge(0.8))
```




```
#checking for normality
shapiro.test(calData$EE.Day)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$EE.Day
## W = 0.9805, p-value = 0.6536
```

```
#check for homogeneity of variance
leveneTest(EE.Day ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  0.3125 0.8162
##       40
```

```
#Run a 2-way ANOVA
#run the multiplicative model to see if there is an interaction term (signif. diff in slope)
my_anova <- aov(EE.Day ~ Diet + Genotype, data = calData)
Anova(my_anova, type = "III") #must run a type III ANOVA because the sample size is not balanced
```

```
## Anova Table (Type III tests)
##
## Response: EE.Day
##              Sum Sq Df   F value    Pr(>F)
## (Intercept) 1561387  1 1261.2974 < 2.2e-16 ***
## Diet          16102  1   13.0072 0.0008342 ***
## Genotype        874  1    0.7064 0.4055243
## Residuals     50755 41
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(my_anova)
```

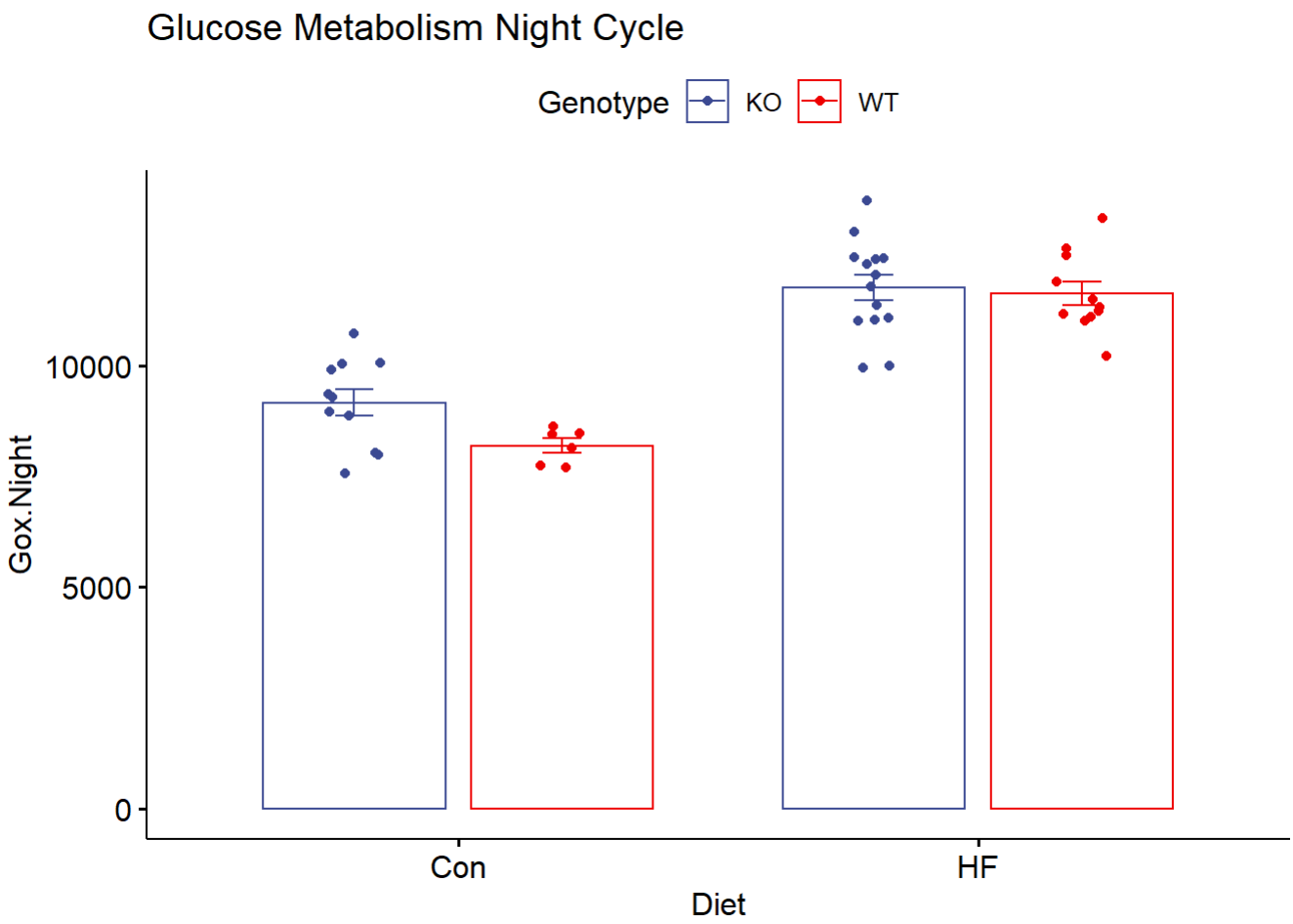
```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = EE.Day ~ Diet + Genotype, data = calData)
##
## $Diet
##            diff      lwr      upr     p adj
## HF-Con 38.62085 16.83359 60.40811 0.0009015
##
## $Genotype
##            diff       lwr      upr     p adj
## WT-KO -9.061789 -30.84905 12.72547 0.4057966
```

No diet-genotype interaction. Only significant difference between diets.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "Gox.Night",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "Glucose Metabolism Night Cycle",
          position = position_dodge(0.8))
```

```
## Warning: Removed 2 rows containing non-finite values (stat_summary).
```

```
## Warning: Removed 2 rows containing missing values (geom_point).
```




```
#checking for normality
shapiro.test(calData$Gox.Night)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$Gox.Night
## W = 0.95728, p-value = 0.118
```

```
#check for homogeneity of variance
leveneTest(Gox.Night ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  1.3118 0.2847
##       38
```

```
#Run a 2-way ANOVA
#run the multiplicative model to see if there is an interaction term (signif. diff in slope)
my_anova <- aov(Gox.Night ~ Diet + Genotype, data = calData)
Anova(my_anova, type = "III") #must run a type III ANOVA because the sample size is not balanced
```

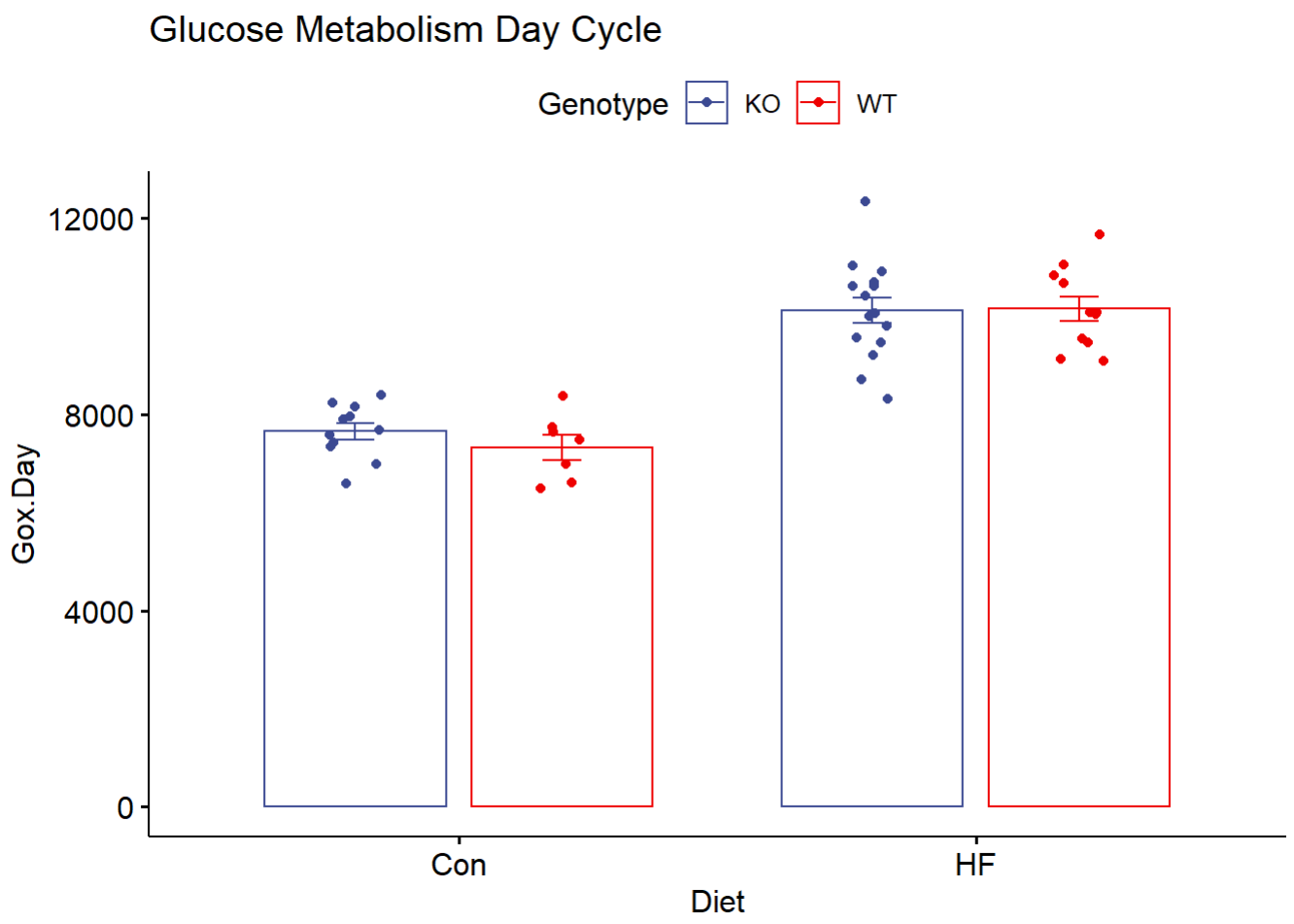
```
## Anova Table (Type III tests)
##
## Response: Gox.Night
##                 Sum Sq Df   F value    Pr(>F)
## (Intercept) 1130909207  1 1243.6562 < 2.2e-16 ***
## Diet          85401002  1   93.9151 6.177e-12 ***
## Genotype       2079267  1    2.2866    0.1386
## Residuals     35464351 39
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(my_anova)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = Gox.Night ~ Diet + Genotype, data = calData)
##
## $Diet
##            diff      lwr      upr p adj
## HF-Con 2876.562 2270.212 3482.912     0
##
## $Genotype
##            diff       lwr      upr    p adj
## WT-KO -451.5785 -1057.929 154.7715 0.140023
```

No diet-genotype interaction. Only significant difference between diets.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "Gox.Day",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "Glucose Metabolism Day Cycle",
          position = position_dodge(0.8))
```




```
#checking for normality
shapiro.test(calData$Gox.Day)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$Gox.Day
## W = 0.96483, p-value = 0.1967
```

```
#check for homogeneity of variance
leveneTest(Gox.Day ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  1.0903 0.3643
##       40
```

```
#Run a 2-way ANOVA
#run the multiplicative model to see if there is an interaction term (signif. diff in slope)
my_anova <- aov(Gox.Day ~ Diet + Genotype, data = calData)
Anova(my_anova, type = "III") #must run a type III ANOVA because the sample size is not balanced
```

```
## Anova Table (Type III tests)
##
## Response: Gox.Day
##                Sum Sq Df   F value    Pr(>F)
## (Intercept) 822275750  1 1241.3528 < 2.2e-16 ***
## Diet         72107468  1  108.8574 4.161e-13 ***
## Genotype       133912  1    0.2022    0.6554
## Residuals    27158521 41
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(my_anova)
```

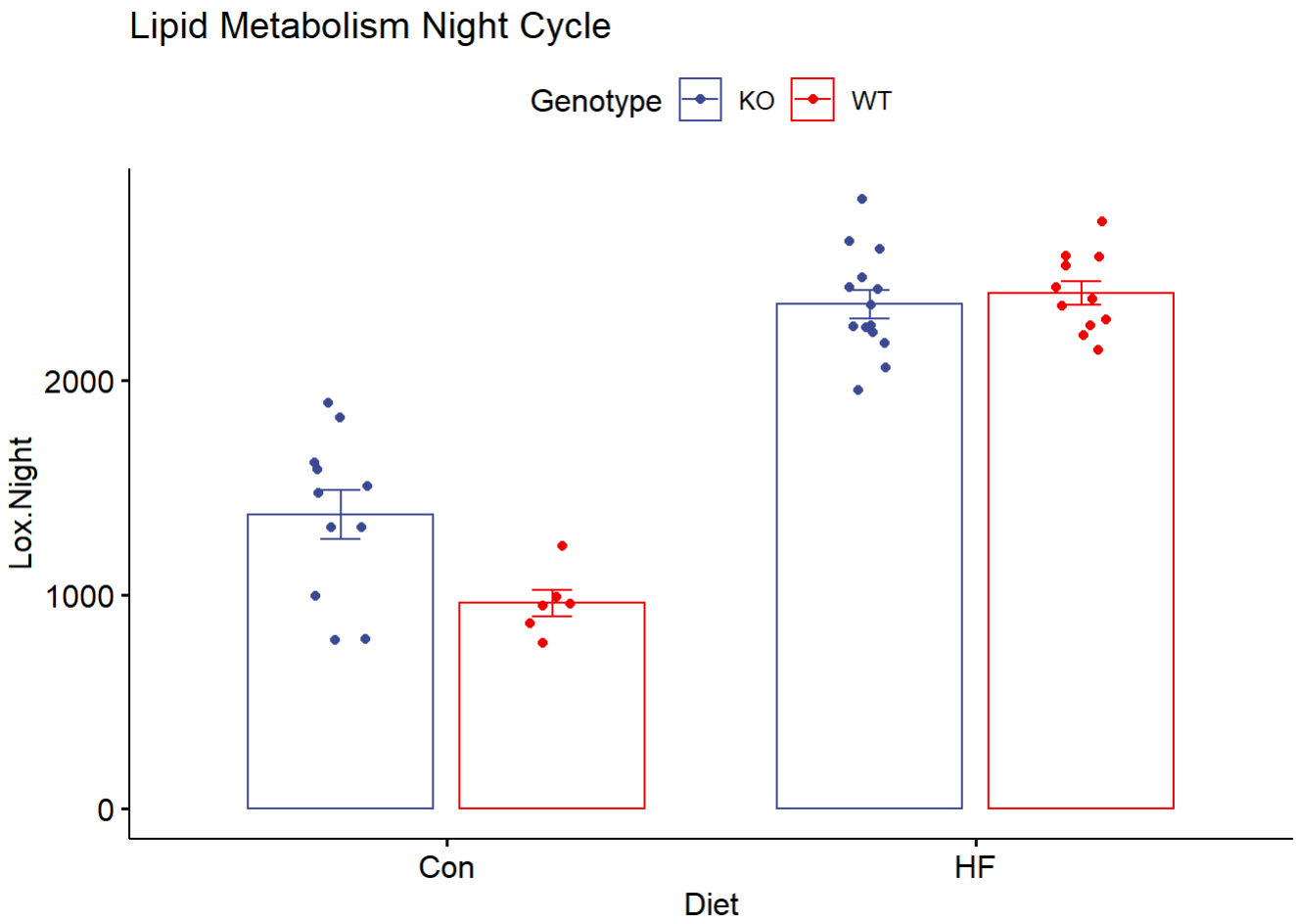
```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = Gox.Day ~ Diet + Genotype, data = calData)
##
## $Diet
##          diff      lwr      upr p adj
## HF-Con 2601.4 2097.416 3105.385     0
##
## $Genotype
##            diff      lwr      upr     p adj
## WT-KO -112.1395 -616.124 391.8451 0.6555389
```

No diet-genotype interaction. Only significant difference between diets.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "Lox.Night",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "Lipid Metabolism Night Cycle",
          position = position_dodge(0.8))
```

```
## Warning: Removed 2 rows containing non-finite values (stat_summary).
```

```
## Warning: Removed 2 rows containing missing values (geom_point).
```




```
#checking for normality
shapiro.test(calData$Lox.Night)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$Lox.Night
## W = 0.89721, p-value = 0.001189
```

```
#check for homogeneity of variance
leveneTest(Lox.Night ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value  Pr(>F)
## group  3  2.2539 0.09777 .
##       38
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#run a wilcoxon test because data was not normal
compare_means(Lox.Night ~ Groups, data = calData, method = "wilcox")
```

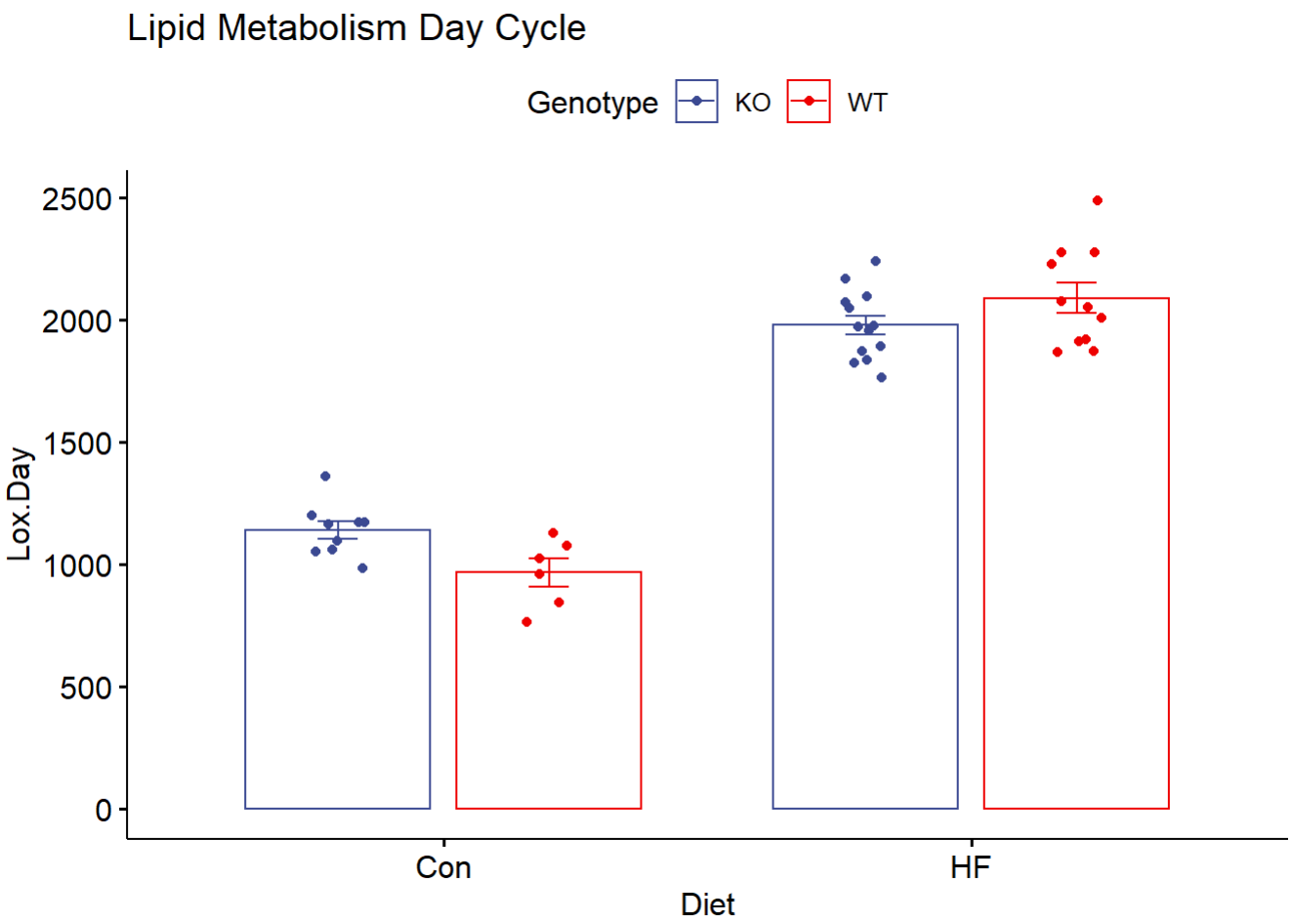
```
## # A tibble: 6 x 8
##   .y.       group1 group2         p   p.adj p.format p.signif method
##   <chr>     <chr>  <chr>      <dbl>   <dbl> <chr>    <chr>    <chr>
## 1 Lox.Night KO_Con KO_HF  0.0000281 0.00017 2.8e-05  ****     Wilcoxon
## 2 Lox.Night KO_Con WT_Con 0.0307    0.061   0.03071  *        Wilcoxon
## 3 Lox.Night KO_Con WT_HF  0.0000815 0.00041 8.2e-05  ****     Wilcoxon
## 4 Lox.Night KO_HF  WT_Con 0.000620  0.0025  0.00062  ***      Wilcoxon
## 5 Lox.Night KO_HF  WT_HF  0.565     0.570   0.56541  ns       Wilcoxon
## 6 Lox.Night WT_Con WT_HF  0.00109   0.0033  0.00109  **       Wilcoxon
```

The data above was not normal therefore we used a wilcoxon test. There is a signgicant difference between KO Con and WT Con. There is a significant increase in lipid metabolism (night cycle) with the control KO mice compared to the WTs (p = 0.03). However this difference is lost upon a high fat diet. This indicates that at baseline the knockout of FPR2 causes the KO mice to have higher lipid metabolism during the night cycle (at times of activity) compared to the WT mice, however this effect is lost when the mice are challeged with a HF diet.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "Lox.Day",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "Lipid Metabolism Day Cycle",
          position = position_dodge(0.8))
```

```
## Warning: Removed 5 rows containing non-finite values (stat_summary).
```

```
## Warning: Removed 5 rows containing missing values (geom_point).
```




```
#checking for normality
shapiro.test(calData$Lox.Day)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$Lox.Day
## W = 0.88775, p-value = 0.001002
```

```
#check for homogeneity of variance
leveneTest(Lox.Day ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3   1.378 0.2656
##       35
```

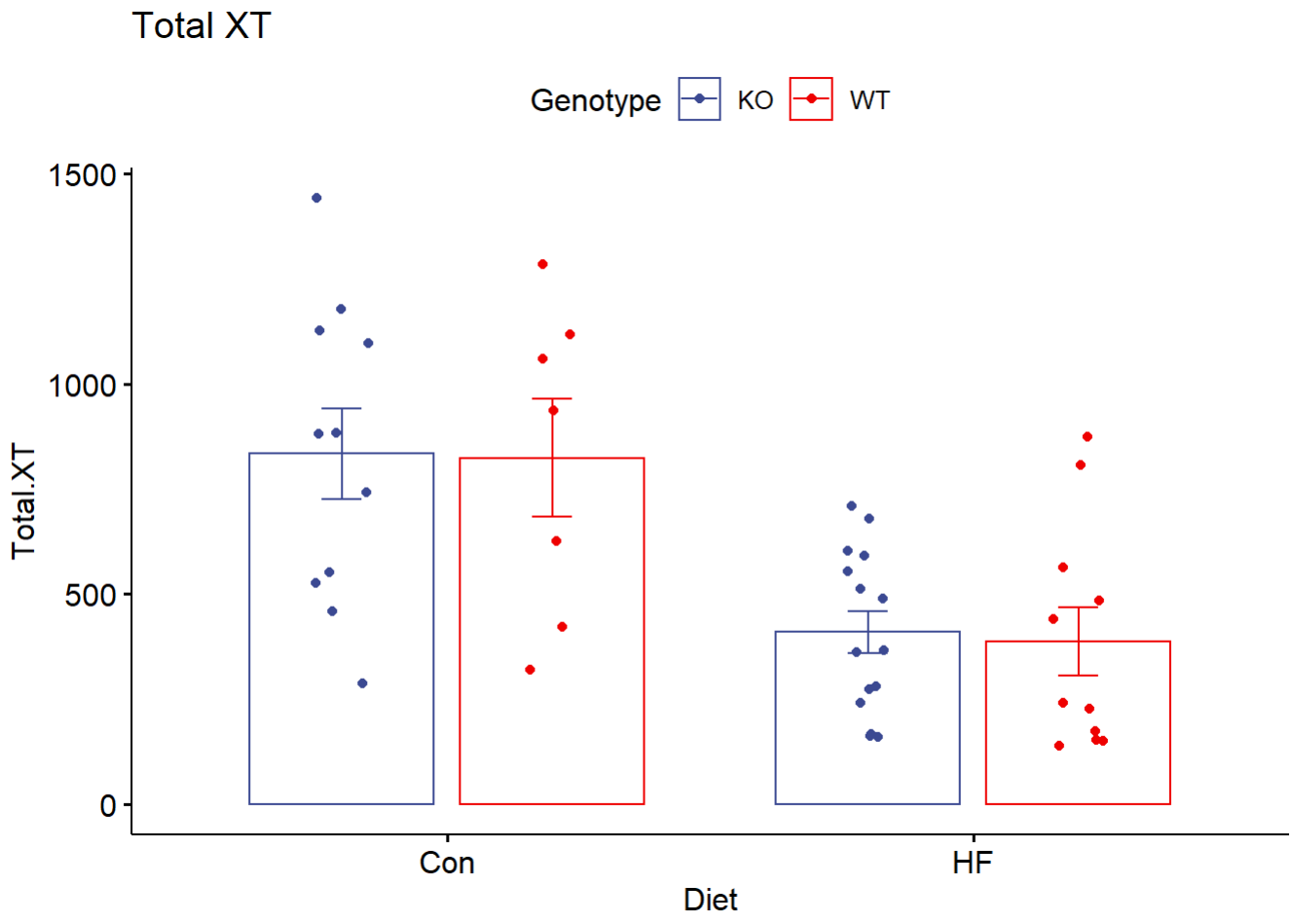
```
#run a wilcoxon test because data was not normal
compare_means(Lox.Day ~ Groups, data = calData, method = "wilcox")
```

```
## # A tibble: 6 x 8
##   .y.     group1 group2        p   p.adj p.format p.signif method
##   <chr>   <chr>  <chr>     <dbl>   <dbl> <chr>    <chr>    <chr>
## 1 Lox.Day KO_Con KO_HF  0.000107 0.00064 0.00011  ***      Wilcoxon
## 2 Lox.Day KO_Con WT_Con 0.0292   0.058   0.02924  *        Wilcoxon
## 3 Lox.Day KO_Con WT_HF  0.000197 0.00099 0.00020  ***      Wilcoxon
## 4 Lox.Day KO_HF  WT_Con 0.000734 0.00290 0.00073  ***      Wilcoxon
## 5 Lox.Day KO_HF  WT_HF  0.183    0.18    0.18268  ns       Wilcoxon
## 6 Lox.Day WT_Con WT_HF  0.00109  0.0033  0.00109  **       Wilcoxon
```

The data above was not normal therefore we used a wilcoxon test. There is a signgicant difference between KO Con and WT Con. There is a significant increase in lipid metabolism (day cycle) with the control KO mice compared to the WTs (p = 0.029). However this difference is lost upon a high fat diet. This indicates that at baseline the knockout of FPR2 causes the KO mice to have higher lipid metabolism during the day cycle (at times of rest) compared to the WT mice, however this effect is lost when the mice are challeged with a HF diet. Although, there is a slight trend with the WT HF mice having elevated lipid metabolism (p = 0.18) - but it is likely not biologically meaningful.

Locomotor Analysis (XT)

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "Total.XT",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "Total XT",
          position = position_dodge(0.8))
```



```
#checking for normality
shapiro.test(calData$Total.XT)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$Total.XT
## W = 0.92854, p-value = 0.009293
```

```
#check for homogeneity of variance
leveneTest(Total.XT ~ Groups, data = calData)
```

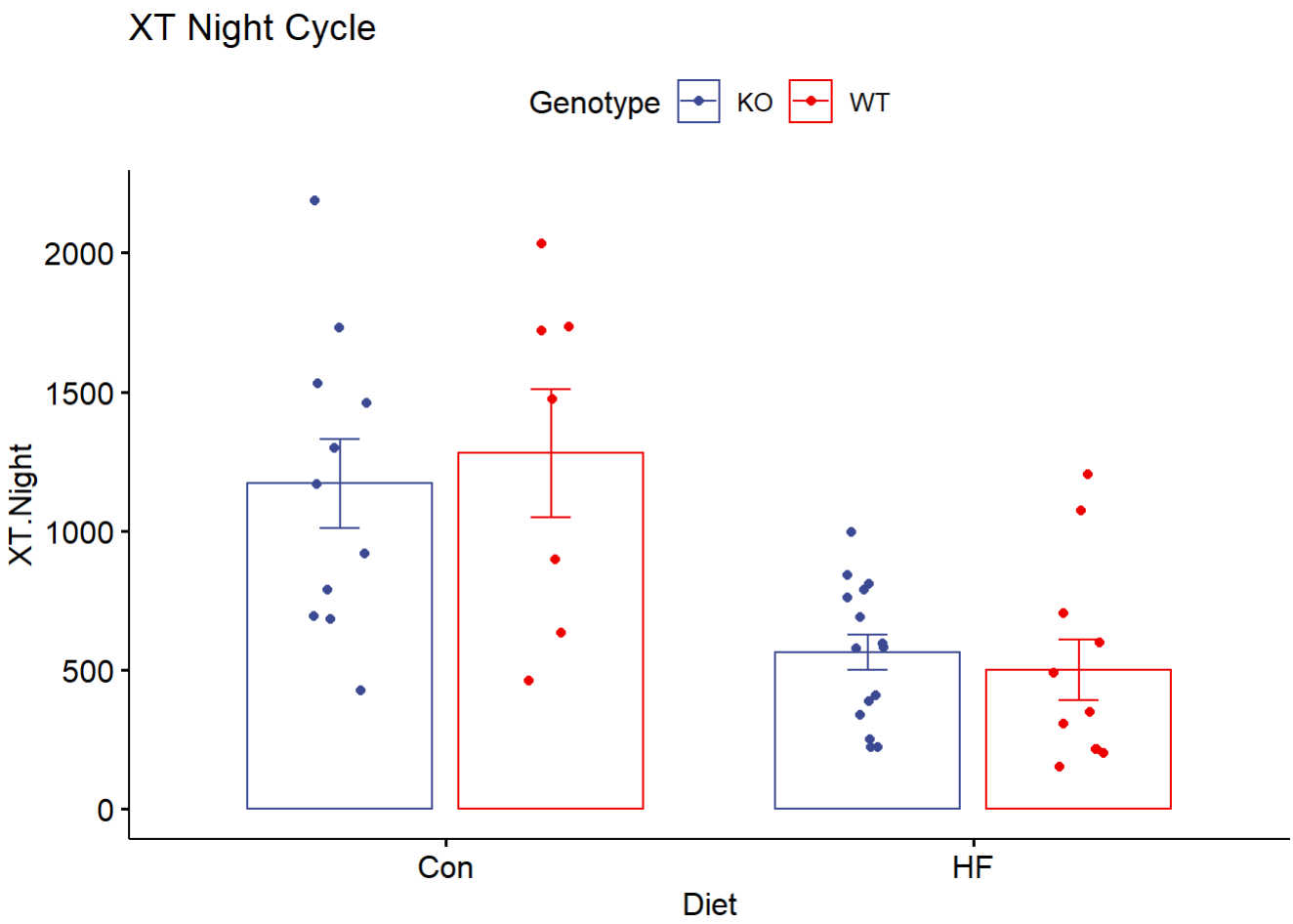
```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  1.4087 0.2544
##       40
```

```
#run a wilcoxon test because data was not normal
compare_means(Total.XT ~ Groups, data = calData, method = "wilcox")
```

```
## # A tibble: 6 x 8
##   .y.      group1 group2       p p.adj p.format p.signif method
##   <chr>    <chr>  <chr>    <dbl> <dbl> <chr>    <chr>    <chr>
## 1 Total.XT KO_Con KO_HF  0.00366 0.022 0.0037   **       Wilcoxon
## 2 Total.XT KO_Con WT_Con 1       1     1.0000   ns       Wilcoxon
## 3 Total.XT KO_Con WT_HF  0.00475 0.024 0.0047   **       Wilcoxon
## 4 Total.XT KO_HF  WT_Con 0.0165  0.066 0.0165   *        Wilcoxon
## 5 Total.XT KO_HF  WT_HF  0.406   0.81  0.4063   ns       Wilcoxon
## 6 Total.XT WT_Con WT_HF  0.0185  0.066 0.0185   *        Wilcoxon
```

Only signficant differences between diet groups. No differences between genotypes.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "XT.Night",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "XT Night Cycle",
          position = position_dodge(0.8))
```




```
#checking for normality
shapiro.test(calData$XT.Night)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$XT.Night
## W = 0.91008, p-value = 0.002255
```

```
#check for homogeneity of variance
leveneTest(XT.Night ~ Groups, data = calData)
```

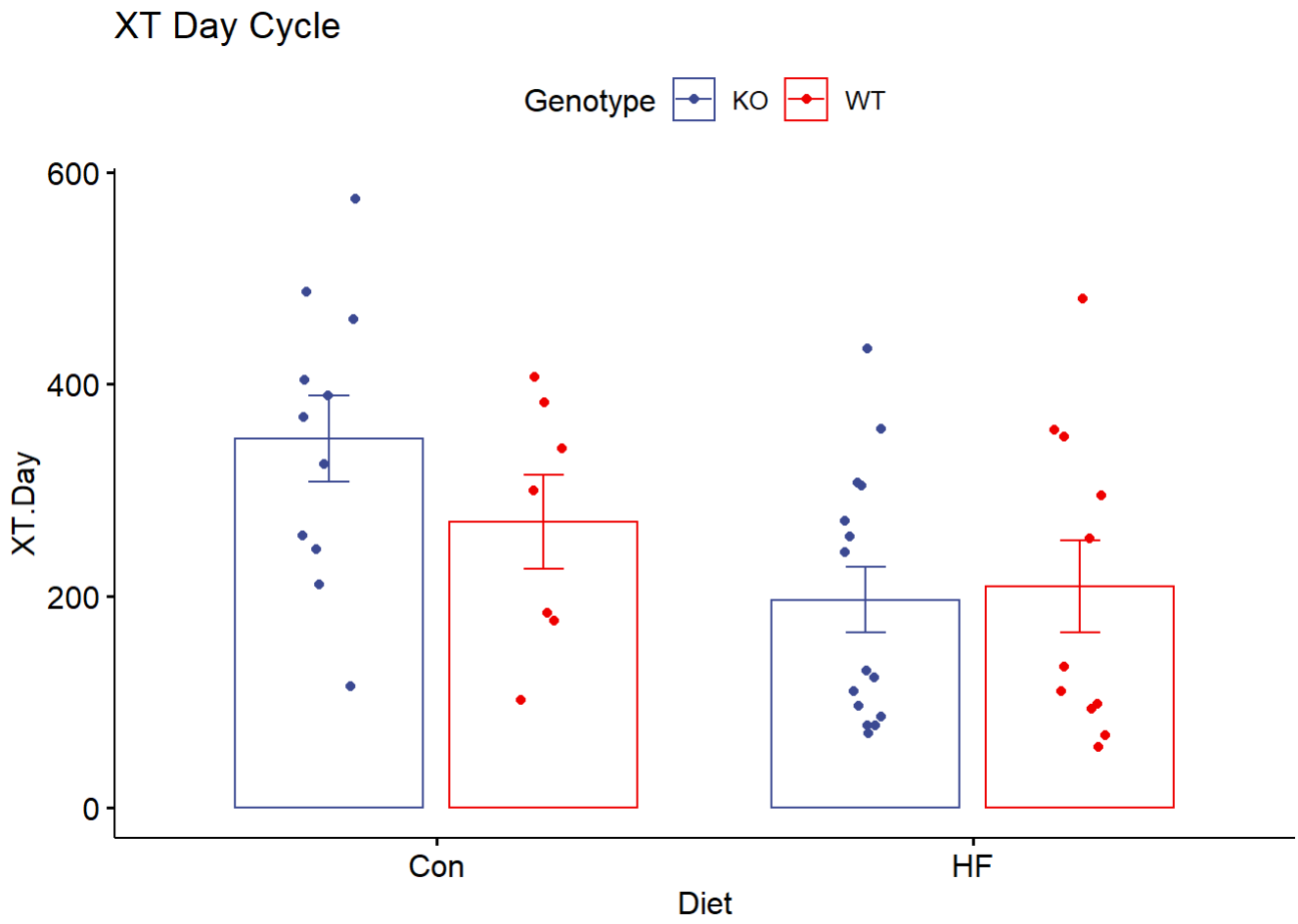
```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value  Pr(>F)
## group  3  2.9888 0.04227 *
##       40
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#run a wilcoxon test because data was not normal
compare_means(XT.Night ~ Groups, data = calData, method = "wilcox")
```

```
## # A tibble: 6 x 8
##   .y.      group1 group2       p p.adj p.format p.signif method
##   <chr>    <chr>  <chr>    <dbl> <dbl> <chr>    <chr>    <chr>
## 1 XT.Night KO_Con KO_HF  0.00261 0.016 0.0026   **       Wilcoxon
## 2 XT.Night KO_Con WT_Con 0.717   0.72  0.7172   ns       Wilcoxon
## 3 XT.Night KO_Con WT_HF  0.00386 0.019 0.0039   **       Wilcoxon
## 4 XT.Night KO_HF  WT_Con 0.0112  0.045 0.0112   *        Wilcoxon
## 5 XT.Night KO_HF  WT_HF  0.299   0.6   0.2993   ns       Wilcoxon
## 6 XT.Night WT_Con WT_HF  0.0112  0.045 0.0112   *        Wilcoxon
```

Only signficant differences between diet groups. No differences between genotypes.

```
# Basic bar plots of means +/- sem with jittered points
ggbarplot(calData, x = "Diet", y = "XT.Day",
          add = c("mean_se", "jitter"),
          color = "Genotype", palette = "aaas", title = "XT Day Cycle",
          position = position_dodge(0.8))
```




```
#checking for normality
shapiro.test(calData$XT.Day)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  calData$XT.Day
## W = 0.93597, p-value = 0.01694
```

```
#check for homogeneity of variance
leveneTest(XT.Day ~ Groups, data = calData)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  0.1263  0.944
##       40
```

```
#run a wilcoxon test because data was not normal
compare_means(XT.Day ~ Groups, data = calData, method = "wilcox")
```

```
## # A tibble: 6 x 8
##   .y.    group1 group2       p p.adj p.format p.signif method
##   <chr>  <chr>  <chr>    <dbl> <dbl> <chr>    <chr>    <chr>
## 1 XT.Day KO_Con KO_HF  0.00946 0.057 0.0095   **       Wilcoxon
## 2 XT.Day KO_Con WT_Con 0.239   0.72  0.2390   ns       Wilcoxon
## 3 XT.Day KO_Con WT_HF  0.0302  0.15  0.0302   *        Wilcoxon
## 4 XT.Day KO_HF  WT_Con 0.159   0.63  0.1586   ns       Wilcoxon
## 5 XT.Day KO_HF  WT_HF  1       1     1.0000   ns       Wilcoxon
## 6 XT.Day WT_Con WT_HF  0.239   0.72  0.2390   ns       Wilcoxon
```

Conclusions

Overall it seems that the FPR2 knockout alters lipid metabolism the most, throughout almost all of the lipid metabolsim calculations/timepoints. However, each time the effect is lost upon a HF diet challenge. This indicates that the gene encoding the FPR2 receptor can affect lipid metabolism under normal dietary conditions; however, in the presence of a HF diet (60% kcal) the dietary challenge of diet-induced obesity masks any effects caused by the knockout.