

STA 440 Case 2

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```
# imports
library(readr)
library(tidyverse)
library(patchwork)
library(scales)
library(ggplot2)
library(dplyr)
library(tidyr)
library(stringr)
library(broom)
library(car)
library(lme4)
library(lmerTest)
```

Background

Mitochondria play a central role in cellular energy metabolism, and alterations in their molecular structures can translate into measurable physiological phenotypes. Characterizing how mitochondrial efficiency changes under different conditions provides important insights into metabolic function and disease mechanisms.

To this end, researchers often measure mitochondrial respiration under a variety of experimental settings. Multiplexed metabolic assay platforms allow simultaneous evaluation of several aspects of mitochondrial functions across substrates, redox conditions, and energetic states. One critical readout is oxygen flux (JO₂), which serves as a direct indicator of mitochondrial respiratory activity. Comparing oxygen flux between experimental groups such as non transgenic and transgenic mice provides a way to evaluate whether genetic differences are associated with altered mitochondrial functions.

Our analysis focuses on modeling and testing for genotype effects on oxygen flux, while accounting for experimental design factors such as substrate type. The aim is to determine whether mitochondrial efficiency differs by genotype, and whether such effects depend on substrate choice or dose.

Data

The data for this study was collected from skeletal muscle mitochondria that are isolated from either non-transgenic or transgenic mice, measured during the mitochondrial energy transduction process. For our model we took into account two main factors: substrate type and dose. The primary factor we are interested in is genotype (non transgenic vs transgenic). Substrate and dose serve as design

factors, and their interactions with genotype are of central interest in assessing whether genetic differences in mitochondrial function vary across energetic states or substrate conditions.

The data we were given originally was in a wide format, so we transformed it into a tidy long format for analysis. We also dropped the basal values since they represent the idle state. Some values were missing so we dropped them.

Model Rationale

Model Implementation and Evaluation

Results

Limitations

Conclusions

```
raw <- read_csv("data/J02.csv", col_names = FALSE, show_col_types = FALSE)

header1 <- as.character(unlist(raw[1, ])) # substrates in row 1
header2 <- as.character(unlist(raw[2, ])) # doses in row 2

sub <- header1
sub[1] <- "Subject"
for (i in 2:length(sub)) {
  if (is.na(sub[i]) || sub[i] == "") sub[i] <- sub[i - 1]
}

dose <- header2
dose[1] <- NA # first col is Subject

new_names <- c("Subject", paste(sub[-1], dose[-1], sep = "_"))

dat <- raw[-c(1, 2), ]
names(dat) <- new_names
dat <- dat |> mutate(across(-Subject, ~ suppressWarnings(as.numeric(.))))

data_long <- dat |>
  pivot_longer(
    cols = -Subject,
    names_to = c("Substrate", "Dose"),
    names_sep = "_",
    values_to = "J02"
  ) |>
  filter(Dose != "Basal") |>
  mutate(
    Genotype = if_else(str_detect(Subject, "^NT"), "NT", "Tg"),
    Dose_num = as.numeric(Dose),
    Dose = factor(Dose,
      levels = c("Basal", "-12.95", "-13.65", "-13.95", "-14.19", "-14.36", "-14.49"),
```

```

ordered = TRUE)
) |>
drop_na(JO2)

glimpse(data_long)

```

```

Rows: 412
Columns: 6
$ Subject   <chr> "NT1", "NT1", "NT1", "NT1", "NT1", "NT1", "NT1", "NT1", "NT1~
$ Substrate <chr> "GM (Glutamate/Malate)", "GM (Glutamate/Malate)", "GM (Gluta~
$ Dose      <ord> -12.95, -13.65, -13.95, -14.19, -14.36, -14.49, -12.95, -13.~
$ JO2       <dbl> 13626.77, 11946.57, 8950.77, 6921.53, 5487.89, 4464.86, 1345~
$ Genotype  <chr> "NT", "NT", "NT", "NT", "NT", "NT", "NT", "NT", "NT", "NT", ~
$ Dose_num  <dbl> -12.95, -13.65, -13.95, -14.19, -14.36, -14.49, -12.95, -13.~

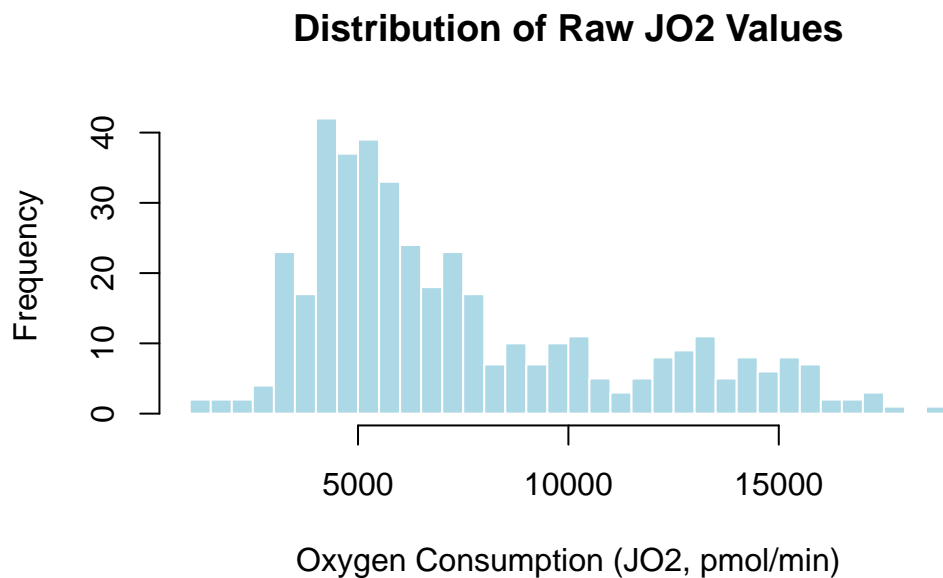
```

Exploratory Data Analysis

```

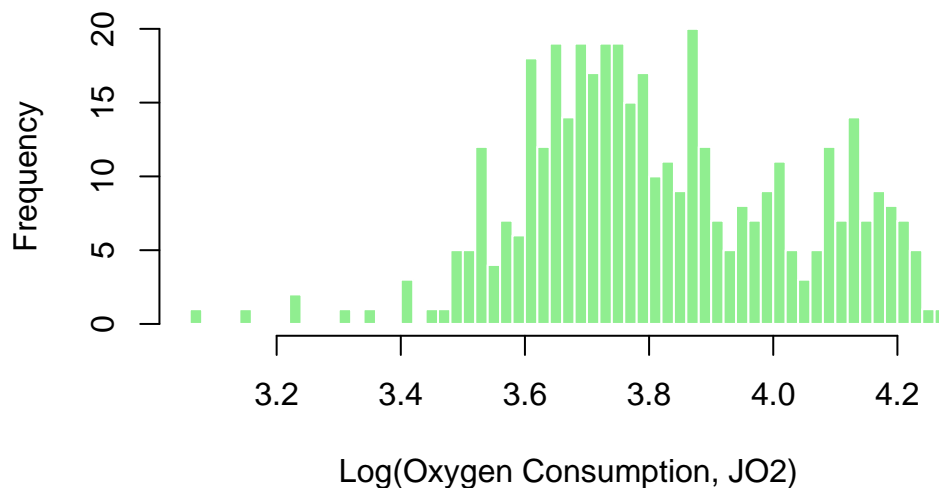
# Raw JO2
hist(
  data_long$JO2,
  breaks = 60,
  main = "Distribution of Raw JO2 Values",
  xlab = "Oxygen Consumption (JO2, pmol/min)",
  ylab = "Frequency",
  col = "lightblue",
  border = "white"
)

```



```
# Log10-transformed JO2
hist(
  log10(data_long$JO2),
  breaks = 60,
  main = "Distribution of Log Transformed JO2 Values",
  xlab = "Log(Oxygen Consumption, JO2)",
  ylab = "Frequency",
  col = "lightgreen",
  border = "white"
)
```

Distribution of Log Transformed JO2 Values



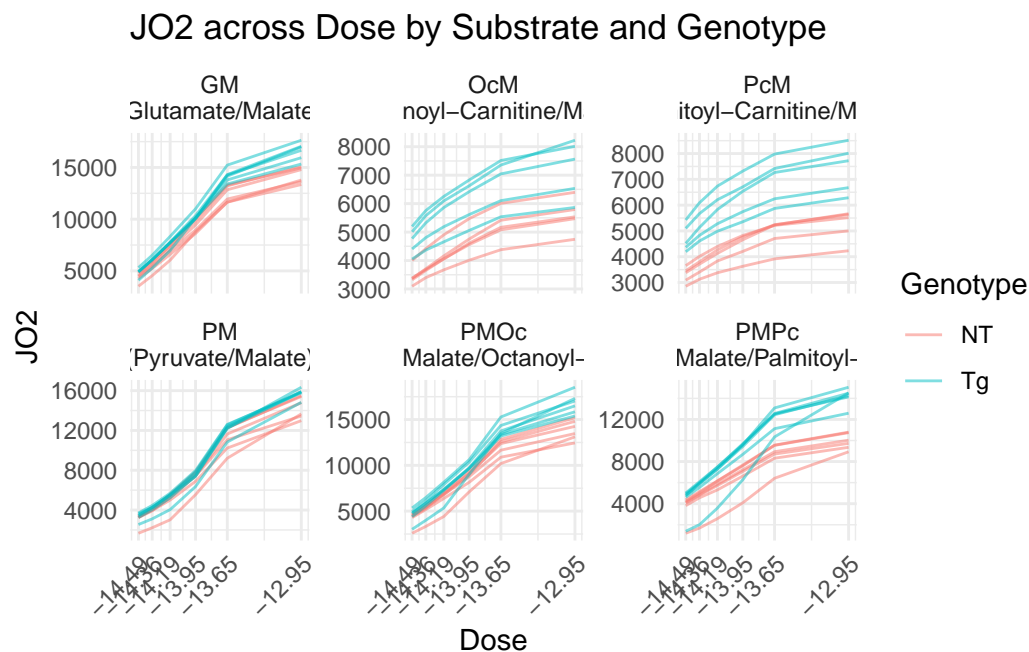
```
data_long <- data_long |>
  mutate(logJO2 = log10(JO2))

# Keep Dose numeric
data_long <- data_long |>
  mutate(Dose = as.numeric(as.character(Dose)))

# Get all unique doses
all_doses <- sort(unique(data_long$Dose))

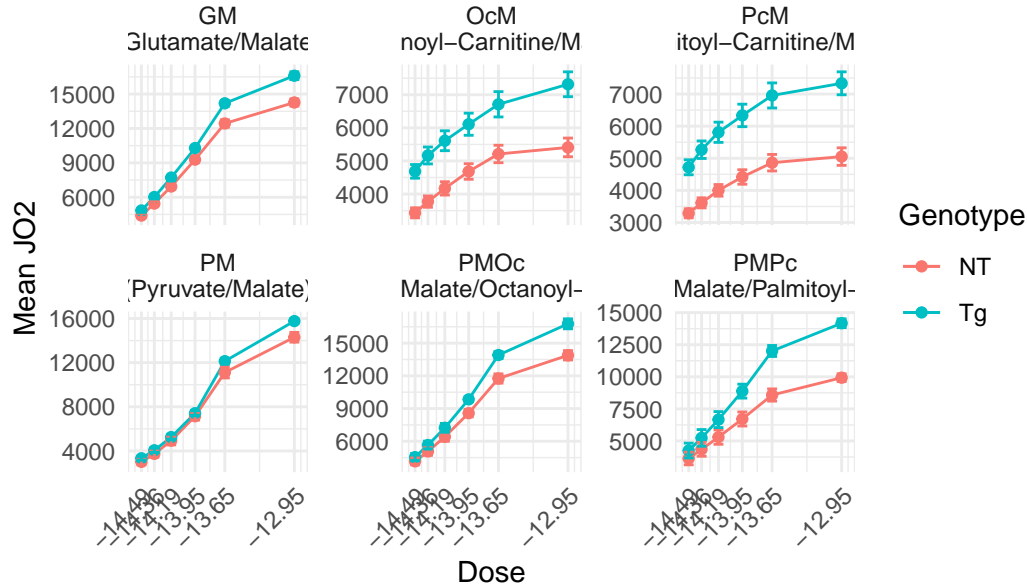
summary_data <- data_long |>
  group_by(Substrate, Genotype, Dose) |>
  summarise(
    mean_JO2 = mean(JO2, na.rm = TRUE),
    se = sd(JO2, na.rm = TRUE) / sqrt(n()),
    .groups = "drop"
  )
```

```
# Plot individual lines with exact dose labels
ggplot(data_long, aes(x = Dose, y = J02, color = Genotype, group = Subject)) +
  geom_line(alpha = 0.5) +
  facet_wrap(~ Substrate, scales = "free_y", labeller = label_wrap_gen(width = 15)) +
  scale_x_continuous(breaks = all_doses) + # show all doses exactly
  theme_minimal() +
  labs(title = "J02 across Dose by Substrate and Genotype") +
  theme(
    axis.text.x = element_text(angle = 45, hjust = 1, size = 9),
    strip.text = element_text(size = 9)
  )
)
```

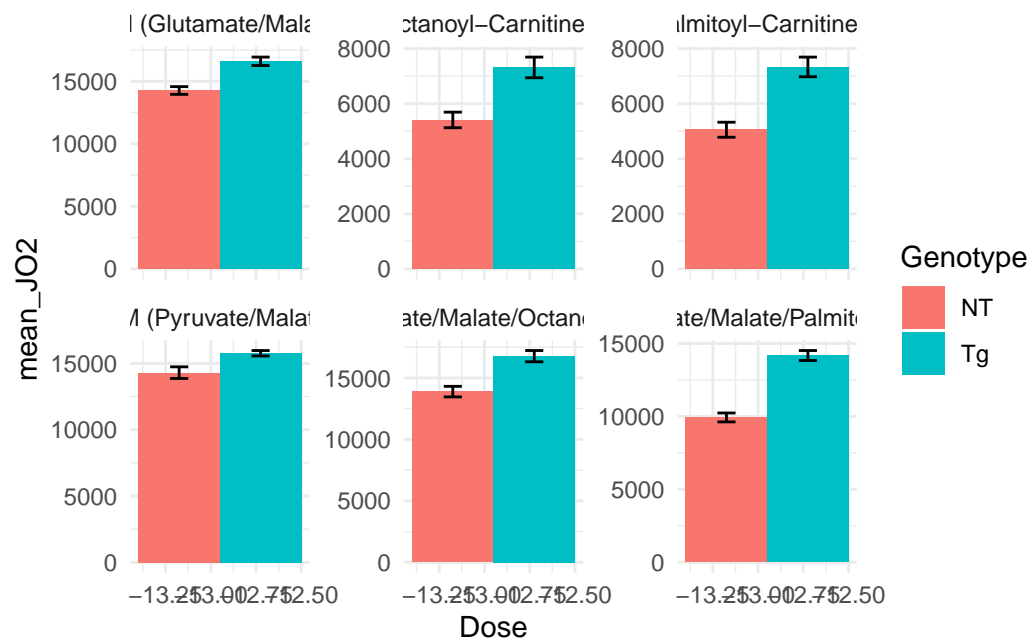


```
# Plot genotype means  $\pm$  SE with exact dose labels
ggplot(summary_data, aes(Dose, mean_J02, color = Genotype, group = Genotype)) +
  geom_line() +
  geom_point() +
  geom_errorbar(aes(ymin = mean_J02 - se, ymax = mean_J02 + se), width = 0.1) +
  facet_wrap(~ Substrate, scales = "free_y", labeller = label_wrap_gen(width = 15)) +
  scale_x_continuous(breaks = all_doses) + # show all doses exactly
  theme_minimal() +
  labs(title = "Mean J02  $\pm$  SE", y = "Mean J02") +
  theme(
    axis.text.x = element_text(angle = 45, hjust = 1, size = 9),
    strip.text = element_text(size = 9)
  )
)
```

Mean JO2 ± SE

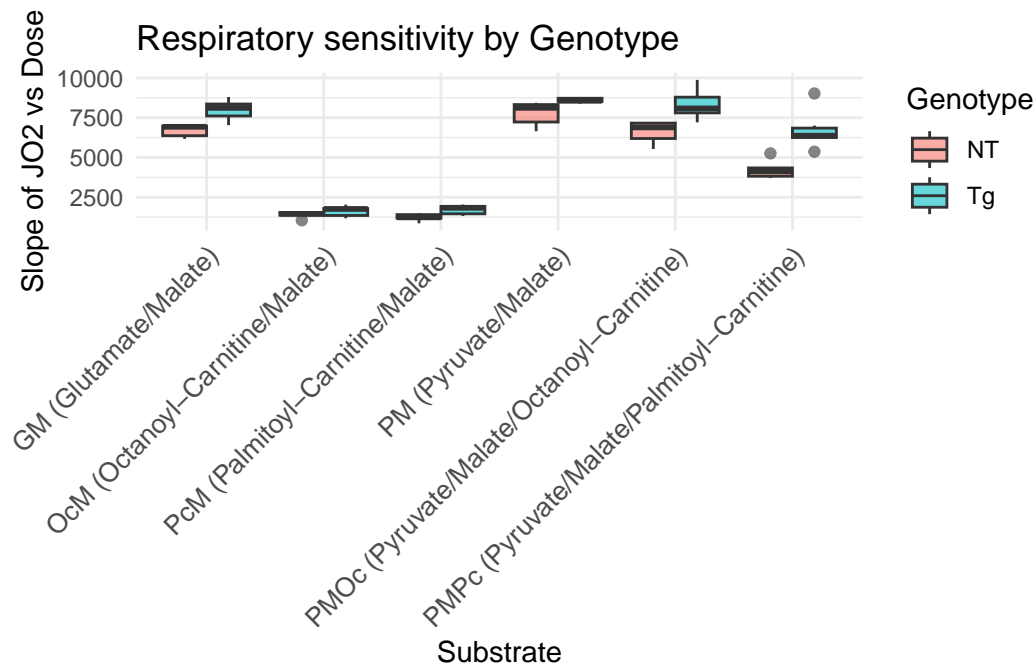


```
data_long |>
  filter(Dose %in% c("Basal", "-12.95")) |>
  group_by(Genotype, Substrate, Dose) |>
  summarise(mean_J02 = mean(J02), se = sd(J02)/sqrt(n()), .groups = "drop") |>
  ggplot(aes(x = Dose, y = mean_J02, fill = Genotype)) +
  geom_col(position = position_dodge()) +
  geom_errorbar(aes(ymin = mean_J02-se, ymax = mean_J02+se,
                    width = 0.2, position = position_dodge(0.9))) +
  facet_wrap(~Substrate, scales = "free_y") +
  theme_minimal()
```



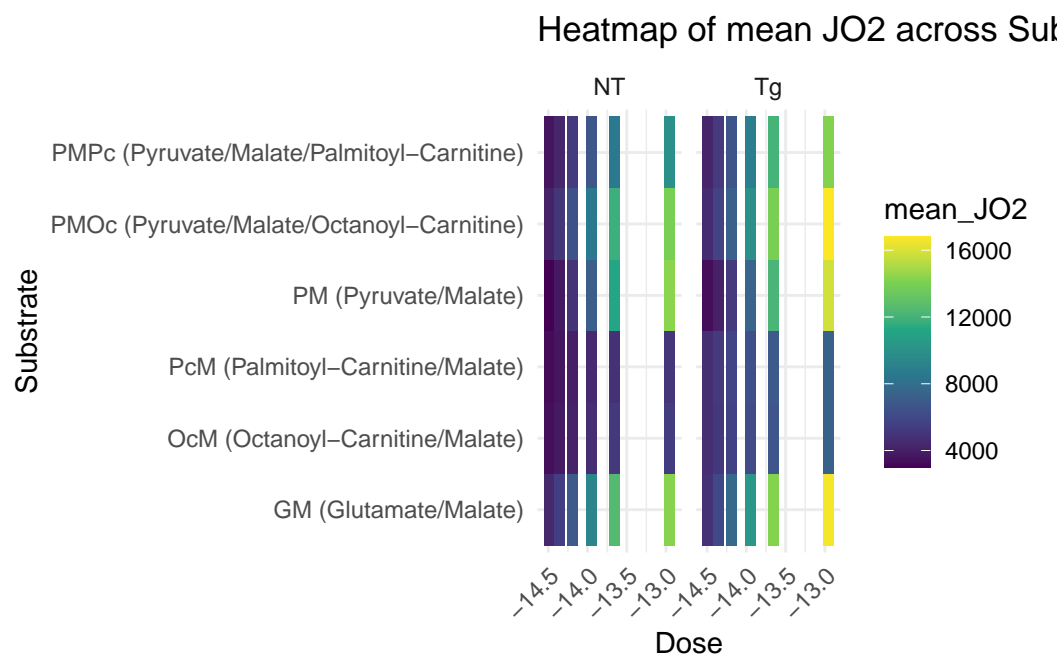
```
slopes <- data_long |>
  group_by(Subject, Genotype, Substrate) |>
  do(tidy(lm(JO2 ~ Dose_num, data = .))) |>
  filter(term == "Dose_num")

ggplot(slopes, aes(x = Substrate, y = estimate, fill = Genotype)) +
  geom_boxplot(alpha = 0.6, position = position_dodge()) +
  theme_minimal() +
  labs(y = "Slope of JO2 vs Dose", title = "Respiratory sensitivity by Genotype") +
  theme(
    axis.text.x = element_text(angle = 45, hjust = 1, size = 10) # rotate labels
  )
```



```
heat <- data_long |>
  group_by(Substrate, Dose, Genotype) |>
  summarise(mean_J02 = mean(J02), .groups = "drop")

ggplot(heat, aes(x = Dose, y = Substrate, fill = mean_J02)) +
  geom_tile() +
  facet_wrap(~Genotype) +
  scale_fill_viridis_c() +
  theme_minimal() +
  labs(title = "Heatmap of mean J02 across Substrates & Doses") +
  theme(
    axis.text.x = element_text(angle = 45, hjust = 1) # rotate x labels
  )
```

Model

```
# Linear model on raw JO2, ignoring subject correlation
lm_raw <- lm(JO2 ~ Substrate * Dose, data = data_long)

# Model summary
summary(lm_raw)
```

Call:

```
lm(formula = JO2 ~ Substrate * Dose, data = data_long)
```

Residuals:

Min	1Q	Median	3Q	Max
-3923.3	-703.6	-51.9	740.8	4192.5

Coefficients:

	Estimate	Std. Error
(Intercept)	111689.1	4332.6
SubstrateOcM (Octanoyl-Carnitine/Malate)	-86257.8	6234.1
SubstratePcM (Palmitoyl-Carnitine/Malate)	-87572.2	6234.1
SubstratePM (Pyruvate/Malate)	10008.0	6127.3
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	1480.8	6127.3
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	-27935.8	6127.3
Dose	7344.5	310.8
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	-5891.0	447.7
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	-5891.5	447.7

```

SubstratePM (Pyruvate/Malate):Dose                839.2      439.5
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose  133.8      439.5
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose -1869.5      439.5
t value Pr(>|t|)
(Intercept)                25.779 < 2e-16 ***
SubstrateOcM (Octanoyl-Carnitine/Malate)          -13.837 < 2e-16 ***
SubstratePcM (Palmitoyl-Carnitine/Malate)          -14.047 < 2e-16 ***
SubstratePM (Pyruvate/Malate)                     1.633  0.1032
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)  0.242  0.8092
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine) -4.559 6.83e-06 ***
Dose                23.633 < 2e-16 ***
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose      -13.159 < 2e-16 ***
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose     -13.361 < 2e-16 ***
SubstratePM (Pyruvate/Malate):Dose                 1.909  0.0569 .
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose  0.304  0.7609
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose -4.254 2.62e-05 ***

```

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1364 on 400 degrees of freedom

Multiple R-squared: 0.8731, Adjusted R-squared: 0.8696

F-statistic: 250.3 on 11 and 400 DF, p-value: < 2.2e-16

```

# ANOVA table (Type I by default)
anova(lm_raw)

```

Analysis of Variance Table

Response: J02

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Substrate	5	1066490422	213298084	114.645	< 2.2e-16 ***
Dose	1	3179528742	3179528742	1708.954	< 2.2e-16 ***
Substrate:Dose	5	875524003	175104801	94.116	< 2.2e-16 ***
Residuals	400	744204872	1860512		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

# If you want Type II/III sums of squares (like in the slides):
Anova(lm_raw, type = 2) # Type II

```

Anova Table (Type II tests)

Response: J02

	Sum Sq	Df	F value	Pr(>F)
Substrate	1160829408	5	124.786	< 2.2e-16 ***
Dose	3179528742	1	1708.954	< 2.2e-16 ***
Substrate:Dose	875524003	5	94.116	< 2.2e-16 ***
Residuals	744204872	400		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```
Anova(lm_raw, type = 3) # Type III
```

Anova Table (Type III tests)

Response: J02

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	1236374890	1	664.535	< 2.2e-16 ***
Substrate	946360045	5	101.731	< 2.2e-16 ***
Dose	1039089121	1	558.496	< 2.2e-16 ***
Substrate:Dose	875524003	5	94.116	< 2.2e-16 ***
Residuals	744204872	400		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```
# Marginal (group-level) model: J02 by genotype only
```

```
lm_marginal <- lm(J02 ~ Genotype, data = data_long)
```

```
# Summary and ANOVA
```

```
summary(lm_marginal)
```

Call:

```
lm(formula = J02 ~ Genotype, data = data_long)
```

Residuals:

Min	1Q	Median	3Q	Max
-6749	-2648	-1325	2207	10396

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	6710.0	258.8	25.928	< 2e-16 ***
GenotypeTg	1424.6	366.0	3.892	0.000116 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 3714 on 410 degrees of freedom

Multiple R-squared: 0.03564, Adjusted R-squared: 0.03329

F-statistic: 15.15 on 1 and 410 DF, p-value: 0.0001158

```
anova(lm_marginal)
```

Analysis of Variance Table

Response: J02

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	1	209039821	209039821	15.151	0.0001158 ***
Residuals	410	5656708219	13796849		

```

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# Multiple regression with genotype, substrate, dose, and interactions
lm_multi <- lm(J02 ~ Genotype * Substrate * Dose, data = data_long)

# Type II/III ANOVA (from car package)
library(car)
Anova(lm_multi, type = 2) # main effects after accounting for others

```

Anova Table (Type II tests)

Response: J02

	Sum Sq	Df	F value	Pr(>F)
Genotype	209039821	1	179.0447	< 2.2e-16 ***
Substrate	1160829408	5	198.8525	< 2.2e-16 ***
Dose	3179528742	1	2723.2989	< 2.2e-16 ***
Genotype:Substrate	24406957	5	4.1810	0.001033 **
Genotype:Dose	41854488	1	35.8488	4.861e-09 ***
Substrate:Dose	875524003	5	149.9791	< 2.2e-16 ***
Genotype:Substrate:Dose	15299858	5	2.6209	0.023970 *
Residuals	453001015	388		

```

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

Anova(lm_multi, type = 3) # tests each term with all interactions

```

Anova Table (Type III tests)

Response: J02

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	516719282	1	442.5753	< 2.2e-16 ***
Genotype	9090126	1	7.7858	0.005526 **
Substrate	424530091	5	72.7229	< 2.2e-16 ***
Dose	432149047	1	370.1401	< 2.2e-16 ***
Genotype:Substrate	15897219	5	2.7232	0.019633 *
Genotype:Dose	8042659	1	6.8886	0.009017 **
Substrate:Dose	388472267	5	66.5461	< 2.2e-16 ***
Genotype:Substrate:Dose	15299858	5	2.6209	0.023970 *
Residuals	453001015	388		

```

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

# Mixed model: random intercept for each mouse
lmm <- lmer(J02 ~ Genotype * Substrate * Dose + (1 | Subject), data = data_long)

# Summary
summary(lmm)

```

Linear mixed model fit by REML. t-tests use Satterthwaite's method [
lmerModLmerTest]

Formula: J02 ~ Genotype * Substrate * Dose + (1 | Subject)

Data: data_long

REML criterion at convergence: 6476.5

Scaled residuals:

Min	1Q	Median	3Q	Max
-2.9474	-0.4988	-0.0847	0.3884	3.3181

Random effects:

Groups	Name	Variance	Std.Dev.
Subject	(Intercept)	491006	700.7
Residual		803156	896.2

Number of obs: 412, groups: Subject, 12

Fixed effects:

	Estimate
(Intercept)	102112.30
GenotypeTg	19153.61
SubstrateOcM (Octanoyl-Carnitine/Malate)	-77851.99
SubstratePcM (Palmitoyl-Carnitine/Malate)	-80193.73
SubstratePM (Pyruvate/Malate)	13562.99
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	-1491.28
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	-36837.04
Dose	6698.31
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate)	-12971.01
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate)	-10916.30
GenotypeTg:SubstratePM (Pyruvate/Malate)	-7110.01
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	5944.17
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	17802.39
GenotypeTg:Dose	1292.30
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	-5259.03
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	-5409.90
SubstratePM (Pyruvate/Malate):Dose	1075.45
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	-72.37
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	-2473.60
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	-959.82
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	-839.15
GenotypeTg:SubstratePM (Pyruvate/Malate):Dose	-472.56
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	412.35
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	1208.17
	Std. Error
(Intercept)	4035.94
GenotypeTg	5707.68
SubstrateOcM (Octanoyl-Carnitine/Malate)	5797.94
SubstratePcM (Palmitoyl-Carnitine/Malate)	5797.94
SubstratePM (Pyruvate/Malate)	5693.32
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	5693.32

SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	5693.32
Dose	288.77
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate)	8199.53
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate)	8199.53
GenotypeTg:SubstratePM (Pyruvate/Malate)	8051.57
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	8051.57
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	8051.57
GenotypeTg:Dose	408.38
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	416.44
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	416.44
SubstratePM (Pyruvate/Malate):Dose	408.38
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	408.38
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	408.38
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	588.94
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	588.94
GenotypeTg:SubstratePM (Pyruvate/Malate):Dose	577.54
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	577.54
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	577.54
	df
(Intercept)	381.49
GenotypeTg	381.49
SubstrateOcM (Octanoyl-Carnitine/Malate)	377.91
SubstratePcM (Palmitoyl-Carnitine/Malate)	377.91
SubstratePM (Pyruvate/Malate)	377.82
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	377.82
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	377.82
Dose	377.82
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate)	377.91
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate)	377.91
GenotypeTg:SubstratePM (Pyruvate/Malate)	377.82
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	377.82
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	377.82
GenotypeTg:Dose	377.82
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	377.92
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	377.92
SubstratePM (Pyruvate/Malate):Dose	377.82
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	377.82
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	377.82
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	377.92
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	377.92
GenotypeTg:SubstratePM (Pyruvate/Malate):Dose	377.82
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	377.82
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	377.82
	t value
(Intercept)	25.301
GenotypeTg	3.356
SubstrateOcM (Octanoyl-Carnitine/Malate)	-13.428
SubstratePcM (Palmitoyl-Carnitine/Malate)	-13.831

SubstratePM (Pyruvate/Malate)	2.382
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	-0.262
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	-6.470
Dose	23.196
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate)	-1.582
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate)	-1.331
GenotypeTg:SubstratePM (Pyruvate/Malate)	-0.883
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	0.738
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	2.211
GenotypeTg:Dose	3.164
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	-12.628
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	-12.991
SubstratePM (Pyruvate/Malate):Dose	2.633
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	-0.177
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	-6.057
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	-1.630
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	-1.425
GenotypeTg:SubstratePM (Pyruvate/Malate):Dose	-0.818
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	0.714
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	2.092
	Pr(> t)
(Intercept)	< 2e-16
GenotypeTg	0.000871
SubstrateOcM (Octanoyl-Carnitine/Malate)	< 2e-16
SubstratePcM (Palmitoyl-Carnitine/Malate)	< 2e-16
SubstratePM (Pyruvate/Malate)	0.017701
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	0.793515
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	3.04e-10
Dose	< 2e-16
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate)	0.114504
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate)	0.183882
GenotypeTg:SubstratePM (Pyruvate/Malate)	0.377766
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	0.460814
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	0.027631
GenotypeTg:Dose	0.001680
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	< 2e-16
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	< 2e-16
SubstratePM (Pyruvate/Malate):Dose	0.008799
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	0.859444
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	3.35e-09
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	0.103990
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	0.155027
GenotypeTg:SubstratePM (Pyruvate/Malate):Dose	0.413738
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	0.475682
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	0.037111
(Intercept)	***
GenotypeTg	***

```

SubstrateOcM (Octanoyl-Carnitine/Malate)          ***
SubstratePcM (Palmitoyl-Carnitine/Malate)          ***
SubstratePM (Pyruvate/Malate)                      *
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine) ***
Dose                                                 ***
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate)
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate)
GenotypeTg:SubstratePM (Pyruvate/Malate)
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine) *
GenotypeTg:Dose                                     **
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose       ***
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose       ***
SubstratePM (Pyruvate/Malate):Dose                  **
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose ***
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate):Dose
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate):Dose
GenotypeTg:SubstratePM (Pyruvate/Malate):Dose
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose *
---

```

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

# Type II/III tests for fixed effects
anova(lmm, type = 3) # Type III ANOVA table

```

Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Genotype	45323596	45323596	1	387.69	56.4318	4.053e-13
Substrate	902653022	180530604	5	377.86	224.7764	< 2.2e-16
Dose	3118509008	3118509008	1	377.96	3882.8166	< 2.2e-16
Genotype:Substrate	16458293	3291659	5	377.86	4.0984	0.001231
Genotype:Dose	39384081	39384081	1	377.96	49.0366	1.155e-11
Substrate:Dose	829135949	165827190	5	377.87	206.4694	< 2.2e-16
Genotype:Substrate:Dose	15927206	3185441	5	377.87	3.9662	0.001616

```

Genotype          ***
Substrate          ***
Dose              ***
Genotype:Substrate **
Genotype:Dose      ***
Substrate:Dose     ***
Genotype:Substrate:Dose **
---

```

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Results

Conclusion

Limitations and Future Work

Appendix