STA 440 Case 2

Krish Bansal, Lily Seelig, Anirudh Jain

Background

Mitochondria play a central role in cellular energy metabolism, and alterations in their molecular structures san 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

```
Rows: 340
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> -13.65, -13.95, -14.19, -14.36, -14.49, -13.65, -13.95, -14.~
$ JO2       <dbl> 11946.57, 8950.77, 6921.53, 5487.89, 4464.86, 11056.88, 7427~
$ Genotype  <chr> "NT", "NT", "NT", "NT", "NT", "NT", "NT", "NT", "NT", "NT", ~
$ Dose_num  <dbl> -13.65, -13.95, -14.19, -14.36, -14.49, -13.65, -13.95, -14.~
```

Exploratory Data Analysis
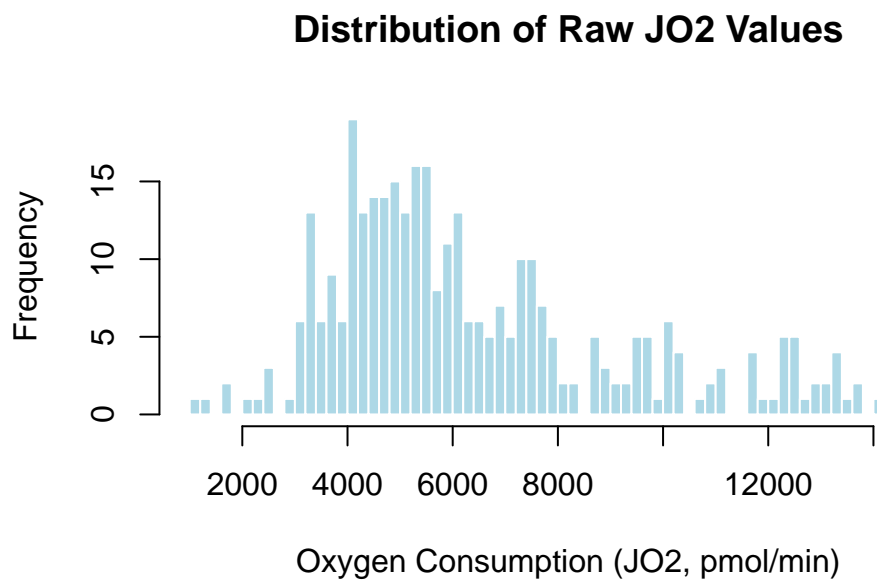
Figure 1: Distribution of raw JO2 values

Model

Linear model on raw JO2 & Diagnostics

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

Distribution of Log Transformed JO2 Values
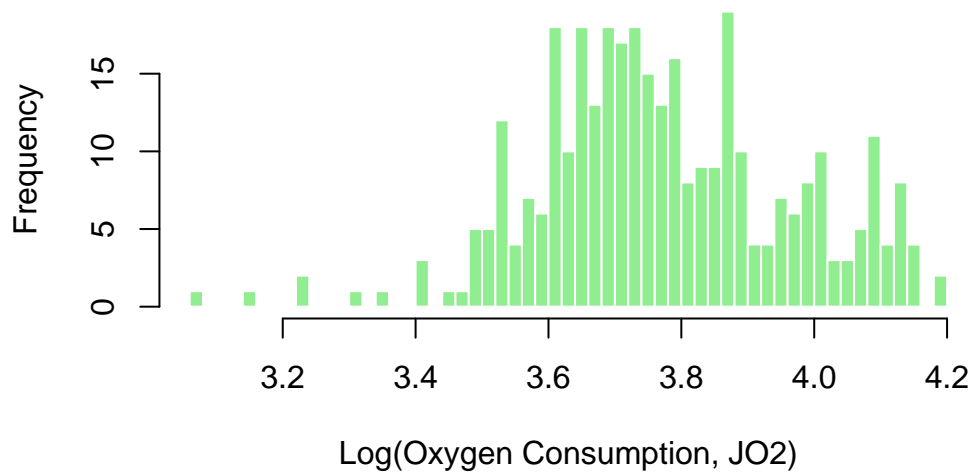
Figure 2: Distribution of log10-transformed JO2 values

JO2 across Dose by Substrate and Genotype
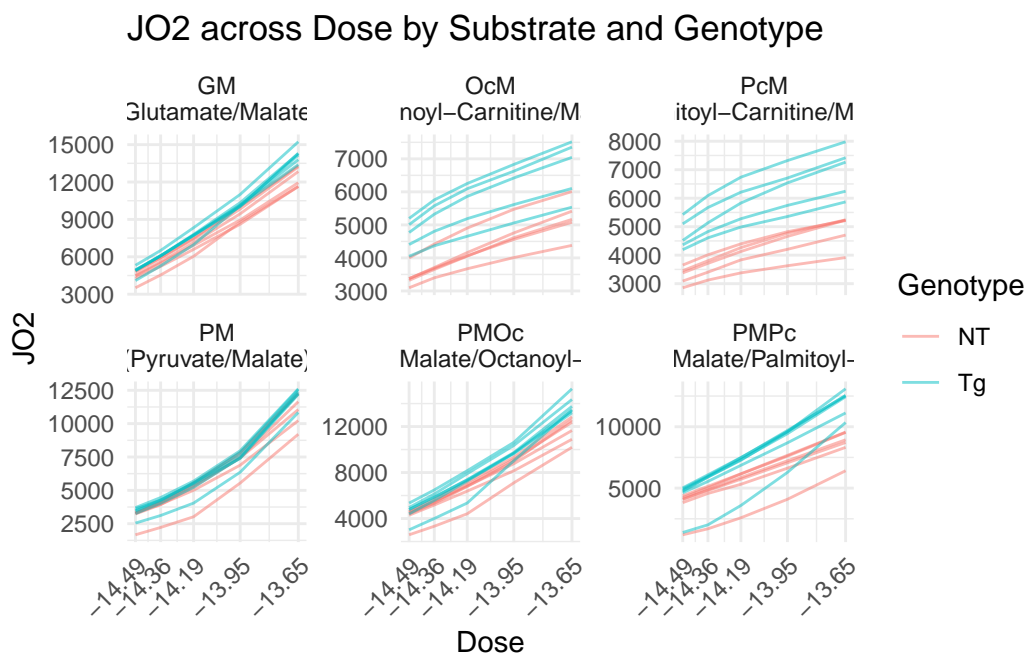
Figure 3: JO2 across dose by substrate and genotype
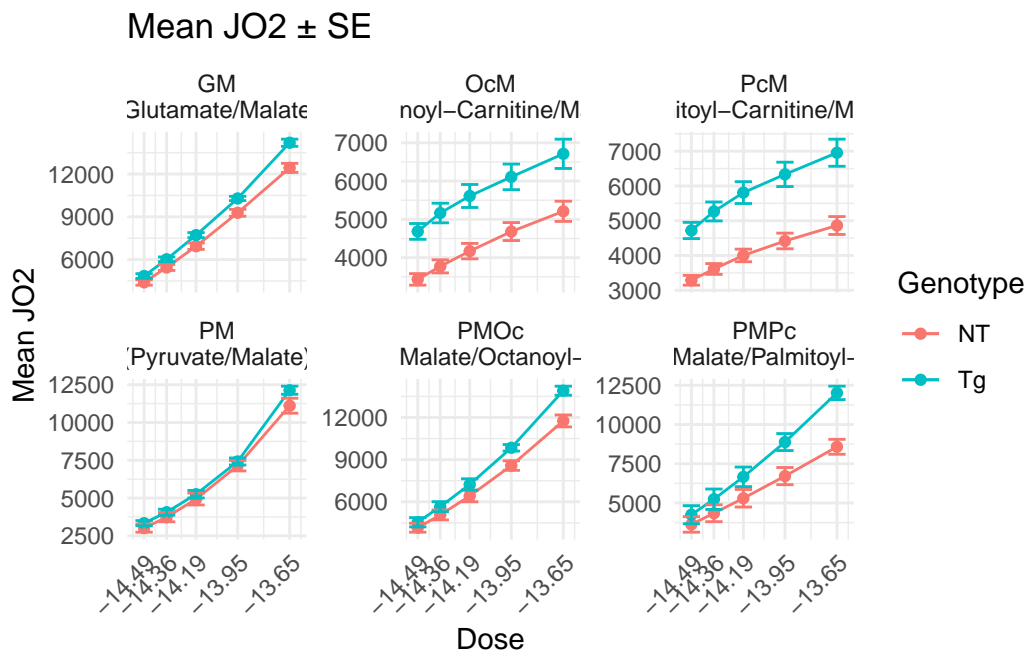
Figure 4: Mean JO2 with ±SE by genotype and substrate
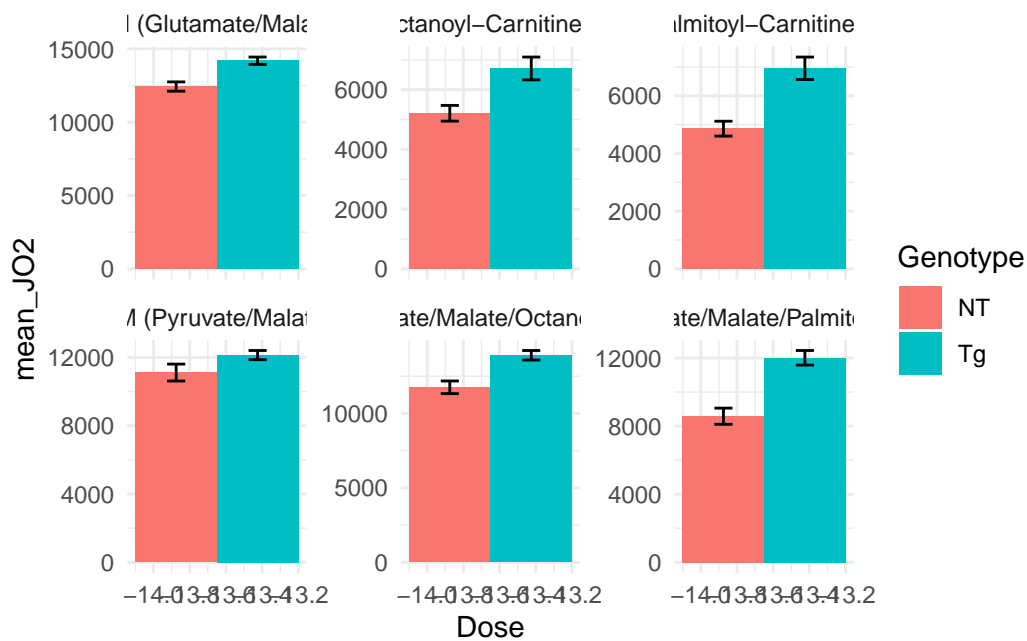
Figure 5: Mean JO2 at Basal and low dose (-13.65)
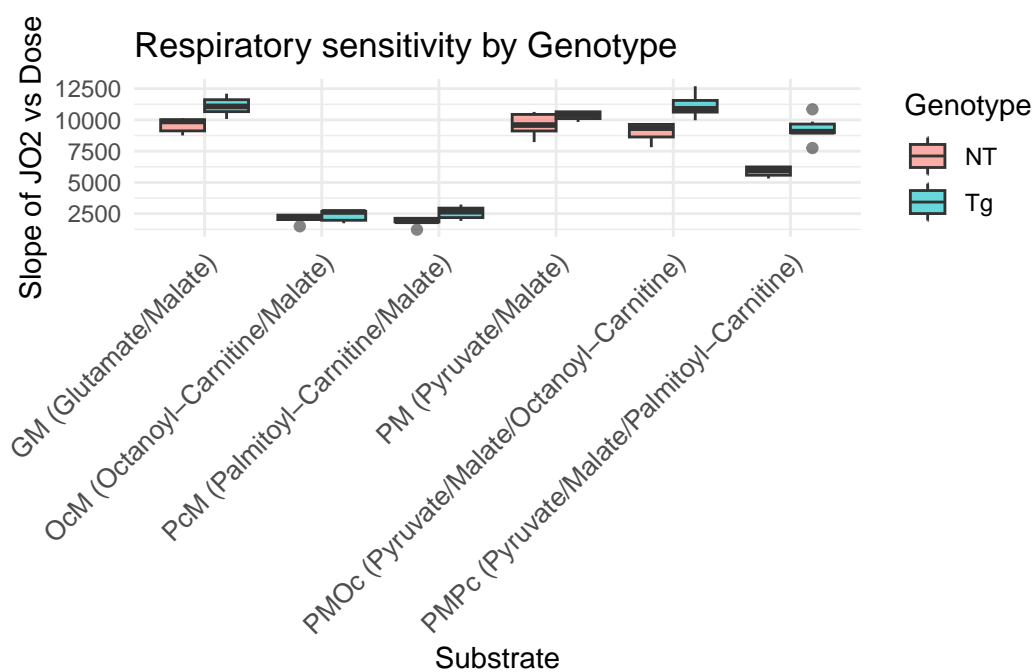
Figure 6: Distribution of JO2–dose slopes by genotype and substrate
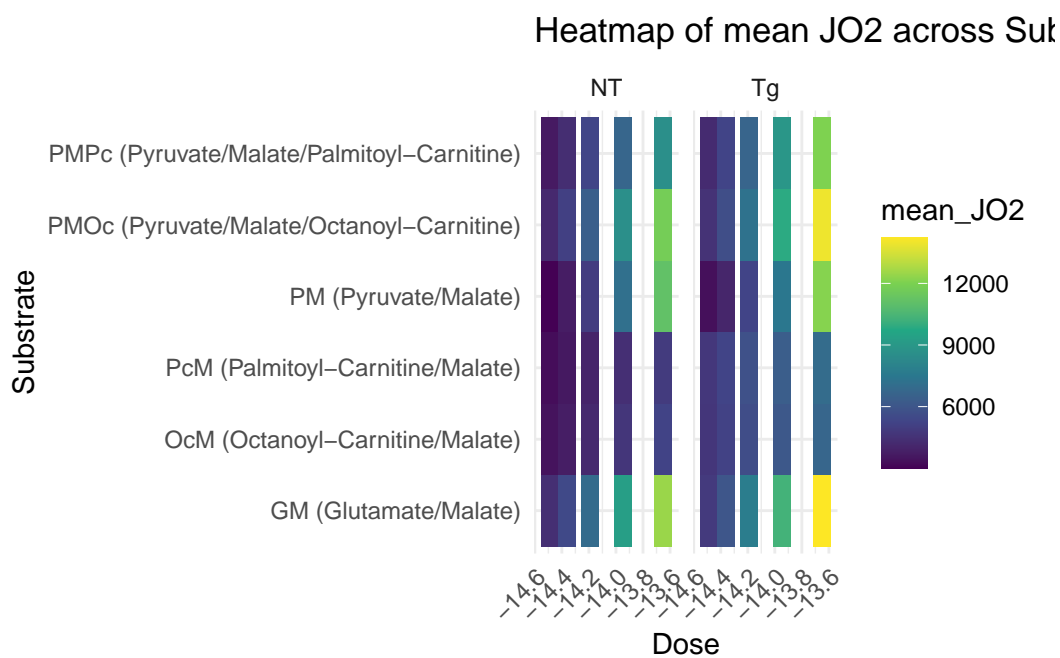
Figure 7: Heatmap of mean JO2 across substrates and doses

```
Residuals:
    Min      1Q  Median      3Q     Max
-3822.3  -646.5    77.6   734.4  2906.7


Coefficients:
                                                           Estimate Std. Error
(Intercept)                                                154427.1     6618.4
SubstrateOcM (Octanoyl-Carnitine/Malate)                  -117987.5     9816.7
SubstratePcM (Palmitoyl-Carnitine/Malate)                 -118080.4     9816.7
SubstratePM (Pyruvate/Malate)                               -7154.0     9359.9
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)          -4072.1     9359.9
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)        -40862.8     9359.9
Dose                                                        10353.3      468.4
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose              -8124.8      694.7
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose             -8129.3      694.7
SubstratePM (Pyruvate/Malate):Dose                          -369.1      662.4
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose     -257.1      662.4
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose   -2779.6      662.4
                                                           t value Pr(>|t|)
(Intercept)                                                 23.333  < 2e-16 ***
SubstrateOcM (Octanoyl-Carnitine/Malate)                   -12.019  < 2e-16 ***
SubstratePcM (Palmitoyl-Carnitine/Malate)                  -12.028  < 2e-16 ***
SubstratePM (Pyruvate/Malate)                               -0.764    0.445
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)          -0.435    0.664
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)         -4.366 1.70e-05 ***
Dose                                                        22.106  < 2e-16 ***
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose              -11.696  < 2e-16 ***
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose             -11.702  < 2e-16 ***
SubstratePM (Pyruvate/Malate):Dose                          -0.557    0.578
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose     -0.388    0.698
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose    -4.197 3.49e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


Residual standard error: 1087 on 328 degrees of freedom
Multiple R-squared:  0.8666,    Adjusted R-squared:  0.8622
F-statistic: 193.8 on 11 and 328 DF,  p-value: < 2.2e-16

Analysis of Variance Table


Response: JO2
               Df      Sum Sq     Mean Sq  F value    Pr(>F)
Substrate       5   501456649   100291330   84.879 < 2.2e-16 ***
Dose            1  1654342978  1654342978 1400.103 < 2.2e-16 ***
Substrate:Dose  5   362539320    72507864   61.365 < 2.2e-16 ***
Residuals     328   387560330     1181586
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Anova Table (Type II tests)

Response: JO2
                  Sum Sq  Df  F value    Pr(>F)
Substrate       501456649   5    84.879 < 2.2e-16 ***
Dose           1654342978   1  1400.103 < 2.2e-16 ***
Substrate:Dose  362539320   5    61.365 < 2.2e-16 ***
Residuals       387560330 328
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Anova Table (Type III tests)

Response: JO2
                  Sum Sq  Df F value    Pr(>F)
(Intercept)     643282533   1 544.423 < 2.2e-16 ***
Substrate       378526362   5  64.071 < 2.2e-16 ***
Dose            577388153   1 488.655 < 2.2e-16 ***
Substrate:Dose  362539320   5  61.365 < 2.2e-16 ***
Residuals       387560330 328
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Marginal Model

```
Call:
lm(formula = JO2 ~ Genotype, data = data_long)

Residuals:
    Min      1Q  Median      3Q     Max
-5720.1 -1974.3  -938.7  1233.3  8178.3

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   5913.0      220.2  26.858  < 2e-16 ***
GenotypeTg    1192.7      311.3   3.831 0.000152 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 2870 on 338 degrees of freedom
Multiple R-squared:  0.04161,   Adjusted R-squared:  0.03878
F-statistic: 14.68 on 1 and 338 DF,  p-value: 0.0001522

Analysis of Variance Table

Response: JO2
           Df     Sum Sq   Mean Sq F value    Pr(>F)
Genotype    1  120919230 120919230  14.675 0.0001522 ***
Residuals 338 2784980047   8239586
---
```

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

Anova Table (Type II tests)


Response: JO2
                          Sum Sq  Df   F value    Pr(>F)
Genotype                120919230   1  171.4797 < 2.2e-16 ***
Substrate               501456649   5  142.2265 < 2.2e-16 ***
Dose                   1654342978   1 2346.0799 < 2.2e-16 ***
Genotype:Substrate       18353582   5    5.2056 0.0001316 ***
Genotype:Dose            17059921   1   24.1933 1.404e-06 ***
Substrate:Dose          362539320   5  102.8259 < 2.2e-16 ***
Genotype:Substrate:Dose   8399558   5    2.3823 0.0384101 *
Residuals               222828039 316
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Anova Table (Type III tests)


Response: JO2
                          Sum Sq  Df  F value  Pr(>F)
(Intercept)             276876709   1 392.6483 < 2e-16 ***
Genotype                  3352741   1   4.7546 0.02996 *
Substrate               170806542   5  48.4453 < 2e-16 ***
Dose                    248030403   1 351.7403 < 2e-16 ***
Genotype:Substrate        8560933   5   2.4281 0.03521 *
Genotype:Dose             3085222   1   4.3753 0.03726 *
Substrate:Dose          162166791   5  45.9948 < 2e-16 ***
Genotype:Substrate:Dose   8399558   5   2.3823 0.03841 *
Residuals               222828039 316
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Mixed Model

```
Linear mixed model fit by REML. t-tests use Satterthwaite's method [
lmerModLmerTest]
Formula: JO2 ~ Genotype * Substrate * Dose + (1 | Subject)
   Data: data_long


REML criterion at convergence: 4950.7


Scaled residuals:
    Min      1Q  Median      3Q     Max
-3.6164 -0.5349 -0.0101  0.5279  3.0060


Random effects:
 Groups   Name        Variance Std.Dev.
 Subject  (Intercept) 629083   793.1
 Residual             278018   527.3
```

```
Number of obs: 340, groups:  Subject, 12

Fixed effects:
                                                                            Estimate
(Intercept)                                                                 143278.42
GenotypeTg                                                                   22297.32
SubstrateOcM (Octanoyl-Carnitine/Malate)                                   -109528.83
SubstratePcM (Palmitoyl-Carnitine/Malate)                                  -113298.52
SubstratePM (Pyruvate/Malate)                                                -1342.39
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)                           -8026.61
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)                         -54350.93
Dose                                                                          9596.47
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate)                         -17511.83
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate)                        -10158.27
GenotypeTg:SubstratePM (Pyruvate/Malate)                                    -11623.31
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)                 7909.05
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)               26976.20
GenotypeTg:Dose                                                               1513.62
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose                                -7485.71
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose                               -7737.11
SubstratePM (Pyruvate/Malate):Dose                                              26.09
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose                       -532.46
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose                     -3706.61
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate):Dose                     -1278.14
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate):Dose                     -784.42
GenotypeTg:SubstratePM (Pyruvate/Malate):Dose                                 -790.30
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose             550.68
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose           1854.02
                                                                           Std. Error
(Intercept)                                                                   4551.72
GenotypeTg                                                                     6437.11
SubstrateOcM (Octanoyl-Carnitine/Malate)                                      6734.22
SubstratePcM (Palmitoyl-Carnitine/Malate)                                     6734.22
SubstratePM (Pyruvate/Malate)                                                 6420.80
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)                            6420.80
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)                           6420.80
Dose                                                                           321.29
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate)                           9523.63
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate)                          9523.63
GenotypeTg:SubstratePM (Pyruvate/Malate)                                      9080.38
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)                 9080.38
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)               9080.38
GenotypeTg:Dose                                                                454.37
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose                                  476.55
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose                                 476.55
SubstratePM (Pyruvate/Malate):Dose                                             454.37
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose                        454.37
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose                       454.37
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GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate):Dose                      673.94
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate):Dose                     673.94
GenotypeTg:SubstratePM (Pyruvate/Malate):Dose                                 642.58
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose            642.58
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose           642.58
                                                                                  df
(Intercept)                                                                   308.85
GenotypeTg                                                                    308.85
SubstrateOcM (Octanoyl-Carnitine/Malate)                                      305.90
SubstratePcM (Palmitoyl-Carnitine/Malate)                                     305.90
SubstratePM (Pyruvate/Malate)                                                 305.90
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)                            305.90
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)                           305.90
Dose                                                                          305.90
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate)                           305.90
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate)                          305.90
GenotypeTg:SubstratePM (Pyruvate/Malate)                                      305.90
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)                 305.90
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)                305.90
GenotypeTg:Dose                                                               305.90
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose                                 305.90
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose                                305.90
SubstratePM (Pyruvate/Malate):Dose                                            305.90
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose                       305.90
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose                      305.90
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate):Dose                      305.90
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate):Dose                     305.90
GenotypeTg:SubstratePM (Pyruvate/Malate):Dose                                 305.90
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose            305.90
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose           305.90
                                                                             t value
(Intercept)                                                                   31.478
GenotypeTg                                                                      3.464
SubstrateOcM (Octanoyl-Carnitine/Malate)                                     -16.265
SubstratePcM (Palmitoyl-Carnitine/Malate)                                    -16.824
SubstratePM (Pyruvate/Malate)                                                 -0.209
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)                            -1.250
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)                           -8.465
Dose                                                                          29.869
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate)                           -1.839
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate)                          -1.067
GenotypeTg:SubstratePM (Pyruvate/Malate)                                       -1.280
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)                  0.871
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)                 2.971
GenotypeTg:Dose                                                                 3.331
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose                                -15.708
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose                               -16.236
SubstratePM (Pyruvate/Malate):Dose                                             0.057
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SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose                       -1.172
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose                      -8.158
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate):Dose                       -1.897
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate):Dose                      -1.164
GenotypeTg:SubstratePM (Pyruvate/Malate):Dose                                  -1.230
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose             0.857
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose            2.885
                                                                              Pr(>|t|)
(Intercept)                                                                   < 2e-16
GenotypeTg                                                                     0.000608
SubstrateOcM (Octanoyl-Carnitine/Malate)                                      < 2e-16
SubstratePcM (Palmitoyl-Carnitine/Malate)                                     < 2e-16
SubstratePM (Pyruvate/Malate)                                                 0.834533
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)                            0.212220
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)                           1.08e-15
Dose                                                                          < 2e-16
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate)                           0.066917
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate)                          0.286976
GenotypeTg:SubstratePM (Pyruvate/Malate)                                      0.201499
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)                 0.384435
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)                0.003205
GenotypeTg:Dose                                                               0.000971
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose                                 < 2e-16
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose                                < 2e-16
SubstratePM (Pyruvate/Malate):Dose                                            0.954247
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose                       0.242161
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose                      8.94e-15
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate):Dose                      0.058835
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate):Dose                     0.245360
GenotypeTg:SubstratePM (Pyruvate/Malate):Dose                                 0.219681
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose  0.392125
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose 0.004188

(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                                 ***
```

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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 III Analysis of Variance Table with Satterthwaite's method
                            Sum Sq      Mean Sq NumDF  DenDF   F value     Pr(>F)
Genotype                   17146031    17146031     1 315.88    61.6724  6.387e-14
Substrate                 380501260    76100252     5 305.90   273.7243  < 2.2e-16
Dose                     1517357360  1517357360     1 305.90  5457.7697  < 2.2e-16
Genotype:Substrate          8543501     1708700     5 305.90     6.1460  1.914e-05
Genotype:Dose              15683854    15683854     1 305.90    56.4131  6.498e-13
Substrate:Dose            362539320    72507864     5 305.90   260.8029  < 2.2e-16
Genotype:Substrate:Dose     8399558     1679912     5 305.90     6.0425  2.370e-05

Genotype                ***
Substrate               ***
Dose                    ***
Genotype:Substrate      ***
Genotype:Dose           ***
Substrate:Dose          ***
Genotype:Substrate:Dose ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

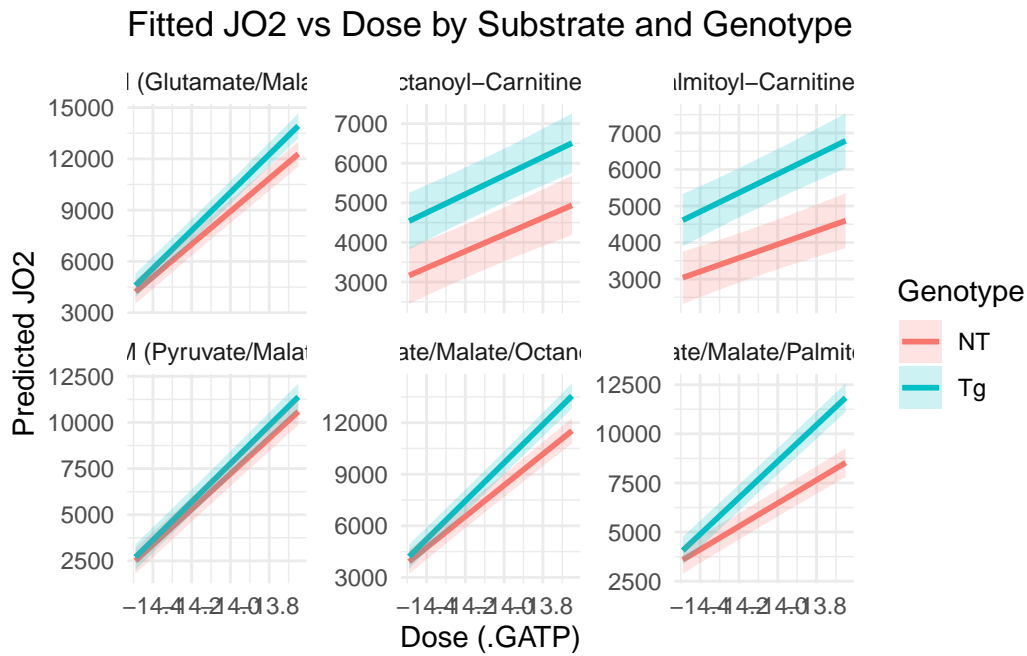# Results

Conclusion

Limitations and Future Work

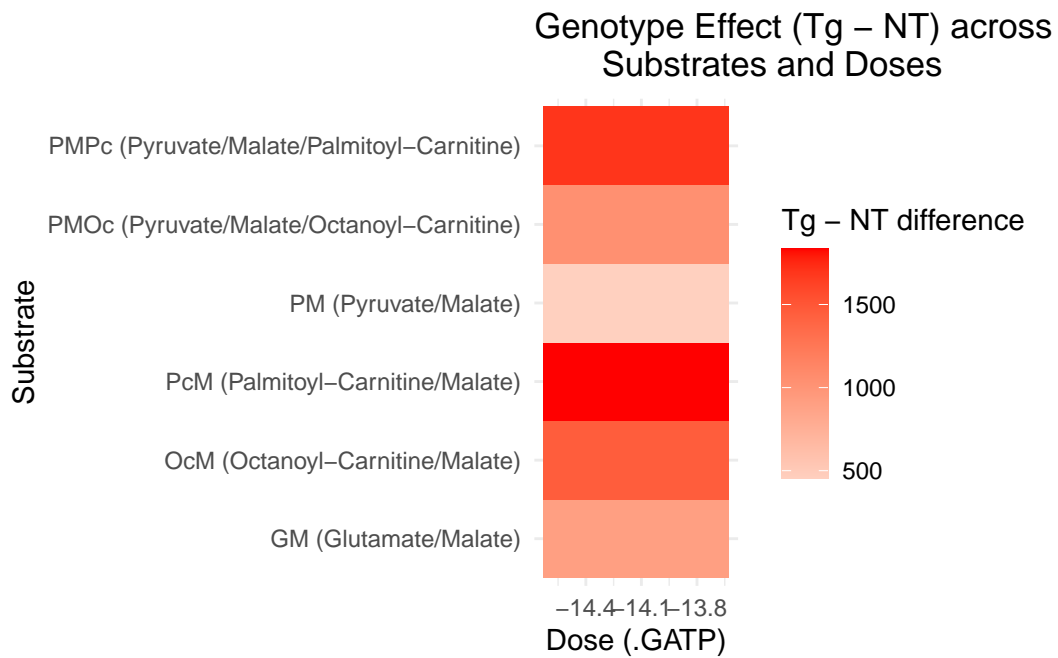Figure 8: Predicted JO2 vs dose by substrate and genotype from mixed model

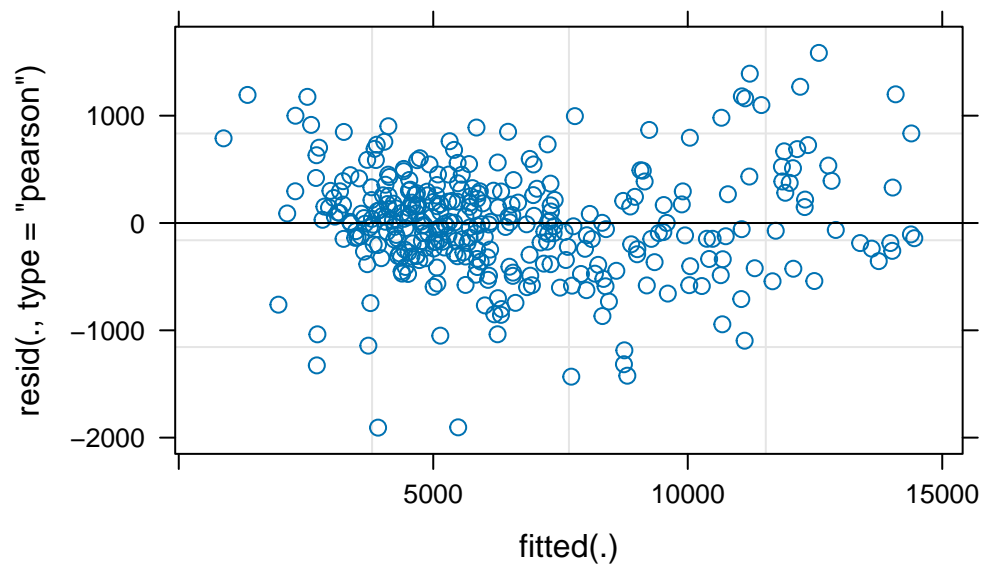Figure 9: Genotype effect (Tg – NT) across substrates and doses
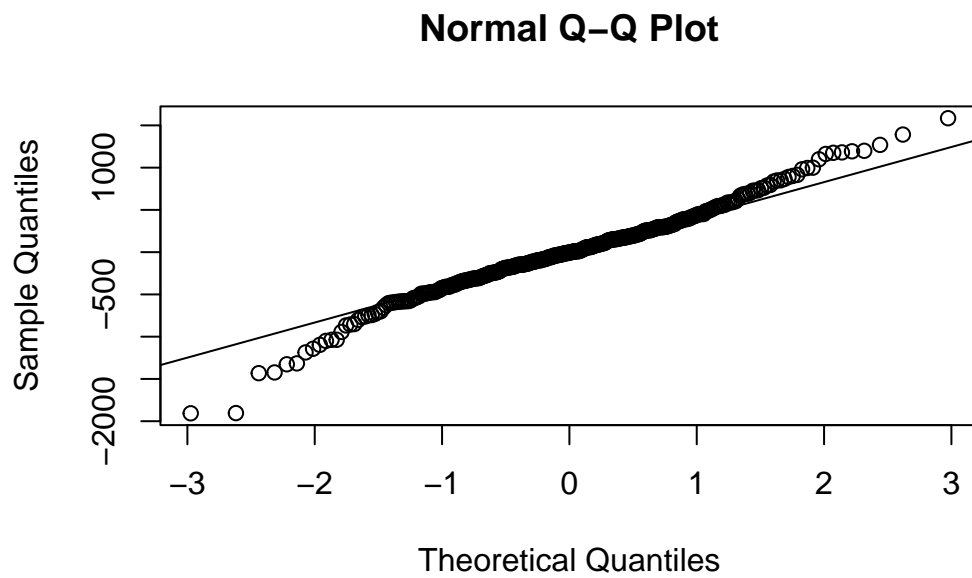
Figure 10: Residuals versus fitted values for mixed model

Normal Q–Q Plot

Figure 11: QQ plot of mixed model residuals

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Appendix