STA 440 Case 2

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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.

Exploratory Data Analysis

Model Rationale

We employed a sequence of models to evaluate the effects of genotype, substrate, and workload (ΔGATP) on oxygen flux (JO). Each model was chosen to address a specific aspect of the experimental design and to progressively incorporate complexity and biological realism.

Baseline model (lm(JO2 ~ Substrate * Dose)

This model considers only substrate and dose effects, ignoring genotype. It serves as a sanity check to establish whether fuel type and workload influence JO as expected. Its main advantage is simplicity and interpretability, but it does not address the central research question concerning genotype differences.

Genotype-only model (lm(JO2 ~ Genotype)

This specification provides a crude comparison of JO between NT and Tg mice by collapsing across all experimental conditions. It is useful as an initial test for an overall genotype effect, but it oversimplifies by ignoring the role of substrate and workload.

Full factorial model (lm(JO2 ~ Genotype * Substrate * Dose)

This model incorporates genotype, substrate, dose, and all interactions. It allows us to examine whether genotype effects vary across substrates or workload levels. The advantage of this approach is that it captures the complexity of the biology, including higher-order interactions. However, it treats each observation as independent and does not account for repeated measures on the same subject, which violates design assumptions.

Mixed-effects model (preferred; lmer(JO2 ~ Genotype * Substrate * Dose + (1 | Subject)

The mixed model includes genotype, substrate, and dose as fixed effects, while adding a random intercept for the subject. This structure accounts for repeated measures within the same mouse and preserves independence across subjects. It balances interpretability with statistical validity, enabling us to test population-level effects while controlling for subject-level variability. Its main limitation is reduced power to detect high-order interactions, given the modest sample size (6 NT, 6 Tg).

While simpler models provided useful descriptive insights, they failed to fully respect the experimental design. The baseline and genotype-only models ignored key sources of variation, while the full factorial model captured interaction effects but treated repeated measures from the same subject as independent, inflating the risk of false positives. The mixed-effects model addressed these shortcomings by incorporating subject-level random effects, thereby accounting for within-mouse correlation while still testing the joint effects of genotype, substrate, and workload at the population level. This framework provides the most statistically rigorous and biologically meaningful analysis of our dataset, balancing model complexity with the need to respect the hierarchical structure of the data.

Model Implementation and Evaluation

We reshaped the raw worksheet into a tidy long format, and restricted analysis to active respiration by removing Basal and the lowest dose (-12.95). We converted dose to a numeric ΔGATP scale for modeling and dropped missing values. Initial checks of distribution and scale (see Figure **?@fig-histograms-1** and **?@fig-histograms-2**) informed the decision to fit models on raw JO2 while using log-transformed views only for EDA.

We then fit a progression of regression models to quantify effects and interactions: - Baseline linear model without genotype: `JO2 ~ Substrate * Dose` (for design checks). - Genotype-only model:

`JO2 ~ Genotype` (coarse group difference). - Full linear model: `JO2 ~ Genotype * Substrate * Dose` (all interactions). - Mixed-effects model with subject intercepts: `JO2 ~ Genotype * Substrate * Dose + (1 | Subject)`.

For the linear models, we report coefficients and ANOVA tables (Table 1, Table 2, Table 3). For the mixed model, we summarize fixed effects (Table 4) and compare overall model fit across candidates using AIC/BIC and log-likelihood (Table 5). Model adequacy was assessed via residual–fitted and normal Q–Q diagnostics (Figure **?@fig-diag-plots-1** and **?@fig-diag-plots-2**). To aid interpretation, we visualized fitted curves with 95% CIs from the mixed model (**?@fig-ggeffects-curves**) and computed estimated marginal means and Tg–NT contrasts across Substrate × Dose cells (**?@fig-emm-heatmap**; Table 6). Supporting exploratory figures show raw trajectories and group means over dose (Figures **?@fig-jo2-dose-plots-1** and **?@fig-jo2-dose-plots-2**), per-subject dose–response slopes (**?@fig-slopes-box**), and mean JO2 heatmaps (**?@fig-heatmap-mean**).

Results

Across substrates, JO2 exhibits a clear dose-dependent response and substantial substrate-to-substrate variability (see trajectories and means in Figures **?@fig-jo2-dose-plots-1** and **?@fig-jo2-dose-plots-2**, and the mean heatmap in **?@fig-heatmap-mean**). The baseline linear model confirms strong effects of Substrate and Dose and their interaction (Table 2). Incorporating Genotype and its interactions in the full linear model indicates that genotype differences are not uniform; they depend on the substrate and/or dose context (Table 3).

Accounting for repeated measures with a subject-level random intercept, the mixed-effects model provides the primary inference. Fixed-effect estimates in Table 4 quantify the average genotype difference at the reference levels and how that difference varies with substrate and dose via interaction terms. The fitted curves from **?@fig-ggeffects-curves** visualize these patterns: separation between Tg and NT varies by substrate and across ΔGATP, with uncertainty bands reflecting the modest sample size. The EMM contrast heatmap and table (**?@fig-emm-heatmap**; Table 6) pinpoint specific Substrate × Dose combinations where Tg differs from NT and summarize effect sizes with standard errors and p-values.

Taken together, the results support dose- and substrate-dependent mitochondrial respiration with evidence of genotype effects that are context-specific. Where significant contrasts appear in Table 6, they align with the visual separation seen in **?@fig-ggeffects-curves**, while diagnostics in Figures **?@fig-diag-plots-1** and **?@fig-diag-plots-2** do not indicate major violations of linear model assumptions. Model comparison in Table 5 favors the mixed-effects specification, balancing improved fit with appropriate handling of within-subject correlation.

Limitations

There are several limitations of this analysis that should be considered when interpreting the results. First, the analysis focused exclusively on the dose-response slopes of the mitochondrial oxygen flux (JO2) after dropping basal condition. Additionally, the analysis is limited to mitochondrial oxygen flux and does not consider other relevant outcomes like reactive oxygen species, membrane potential, and redox state. Also, although the model accounted for some heteroscedasticity, the increasing variance of the JO2 with higher mean values was only partially addressed and may still affect estimates.

The study was also limited by sample size (6 non-transgenic mice and 6 transgenic mice) which reduces the statistical power, particularly for detecting complex interactions or subtle nonlinearities. To account for this, the analysis assumed a linear relationship between the free energy of ATP hydrolysis and JO2 across the studied range. While the assumption appeared reasonable within the available data, nonlinear responses outside of the observed range cannot be ruled out. Finally, the mixed effects modeling approach included random intercepts to capture subject level variability but did not incorporate random slopes, potentially underestimating within subject differences in dose response patterns. However, residuals were approximately normal after accounting for fixed effects, and the assumption of independence across subjects was reasonable given the experimental design.

Conclusions

Appendix

```
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.~
```

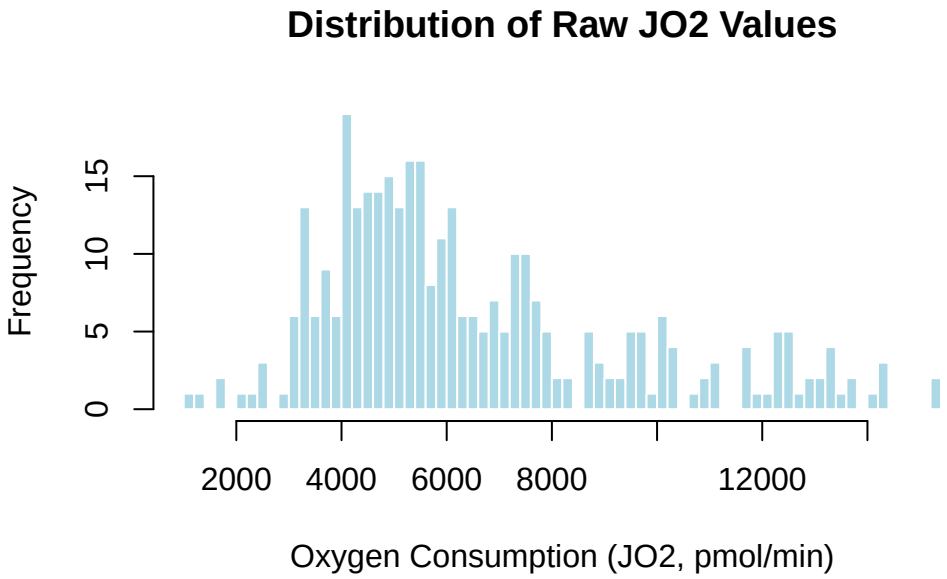

Figure 1: Distribution of raw JO2 values

Linear model on raw JO2 & Diagnostics

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

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
```

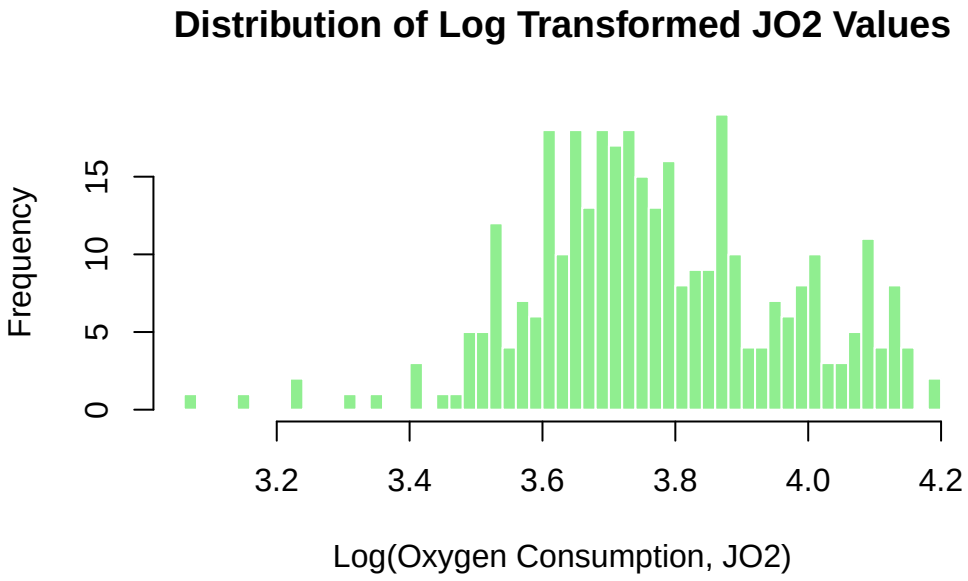

Figure 2: Distribution of log10-transformed JO2 values

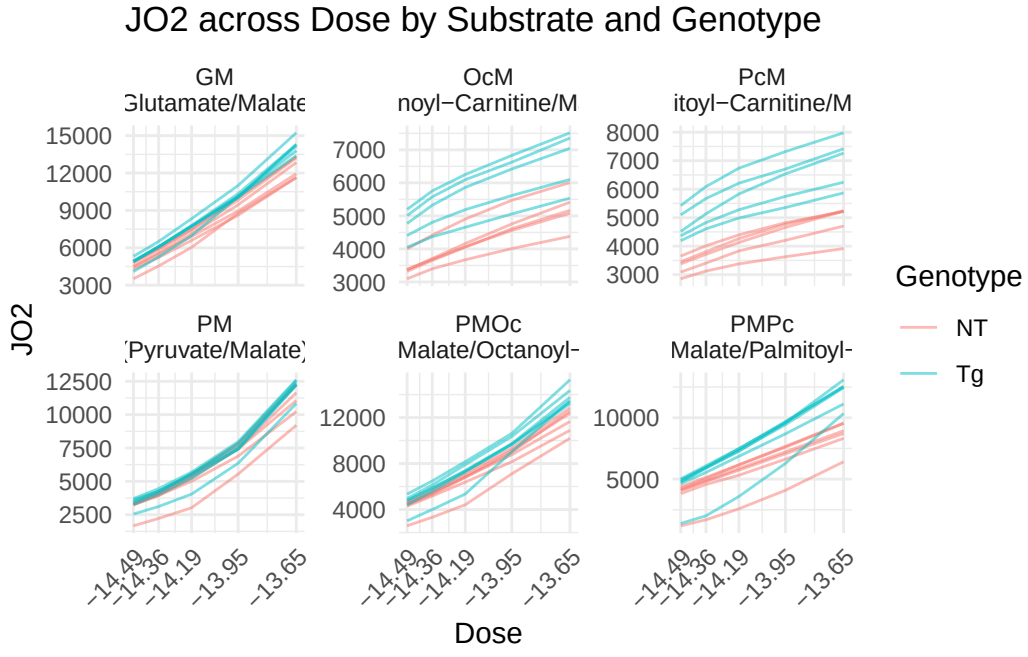

Figure 3: JO2 across dose by substrate and genotype

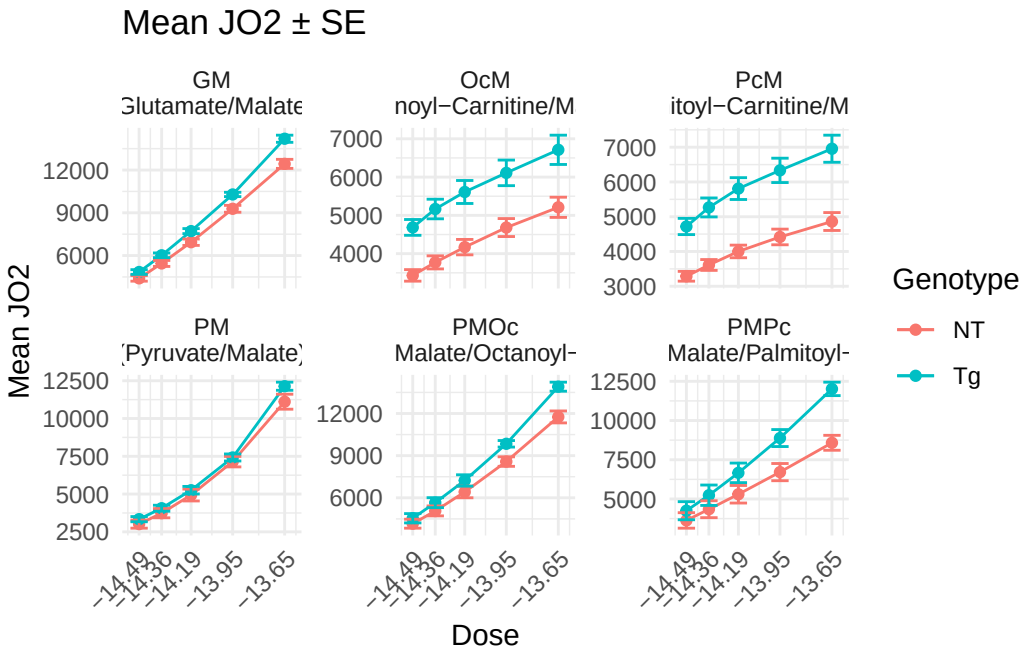

Figure 4: Mean JO2 with ±SE by genotype and substrate

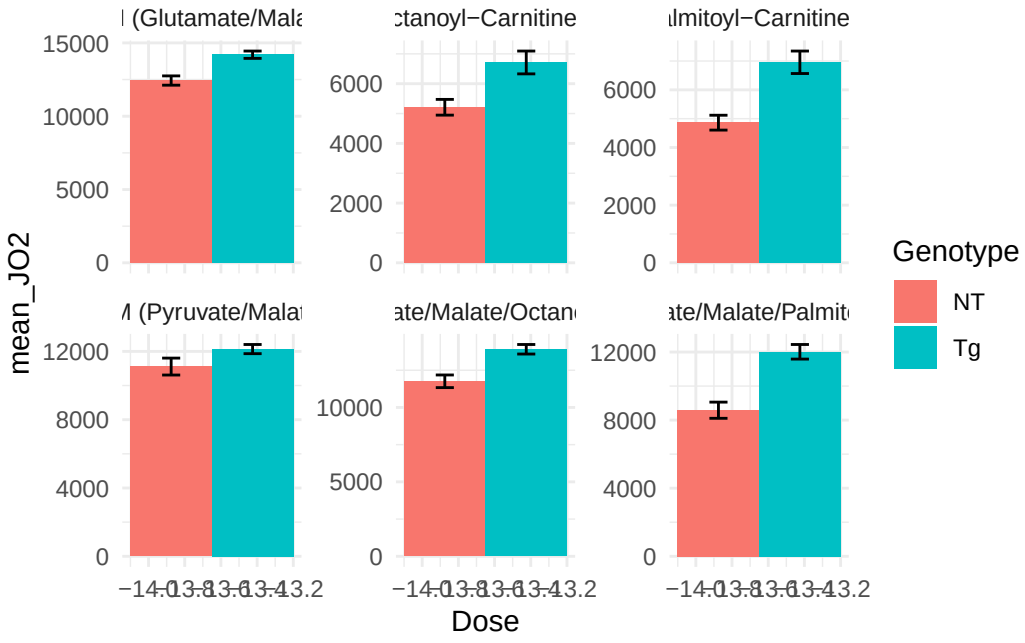

Figure 5: Mean JO2 at Basal and low dose (-13.65)

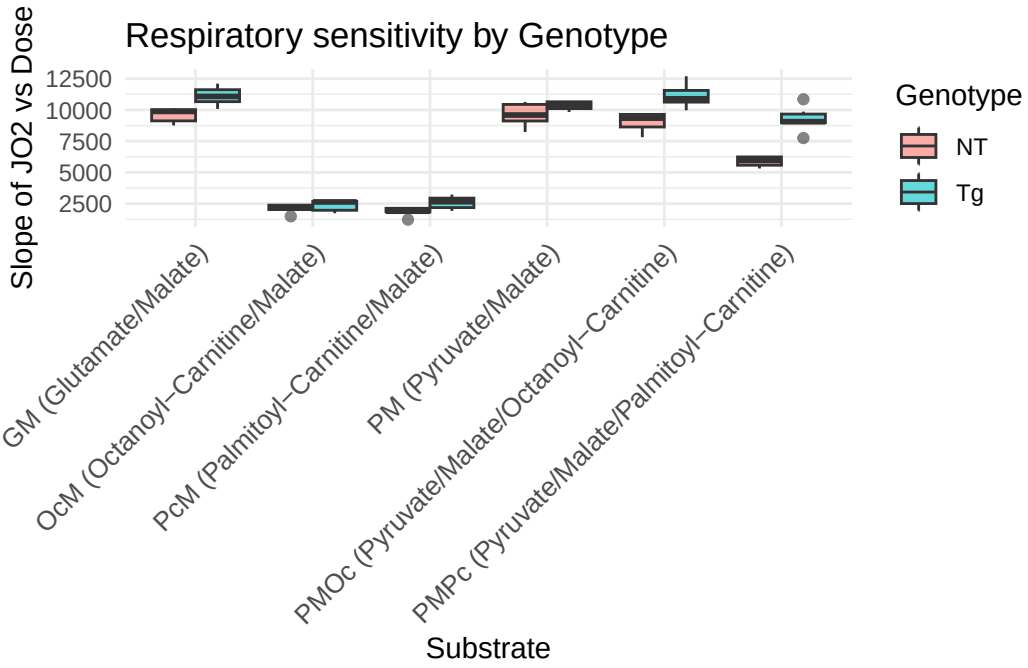

Figure 6: Distribution of JO2–dose slopes by genotype and substrate

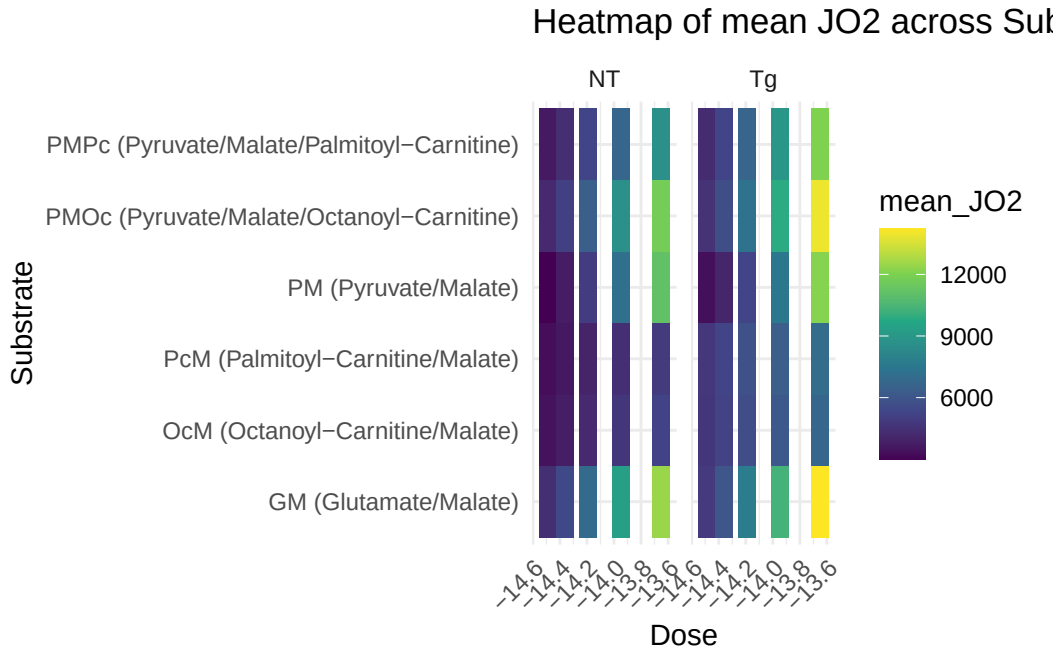

Figure 7: Heatmap of mean JO2 across substrates and doses

```
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
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
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
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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                            ***
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
```

Model Tables

Table 1: Linear model (JO2 ~ Substrate * Dose) coefficients

term	estimate	std.error	statistic	p.value
(Intercept)	154427.081	6618.435	23.333	0.000
SubstrateOcM (Octanoyl-Carnitine/Malate)	-117987.489	9816.726	-12.019	0.000
SubstratePcM (Palmitoyl-Carnitine/Malate)	-118080.399	9816.726	-12.028	0.000
SubstratePM (Pyruvate/Malate)	-7154.046	9359.881	-0.764	0.445
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	-4072.084	9359.881	-0.435	0.664
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	-40862.833	9359.881	-4.366	0.000
Dose	10353.285	468.357	22.106	0.000
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	-8124.780	694.686	-11.696	0.000
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	-8129.319	694.686	-11.702	0.000
SubstratePM (Pyruvate/Malate):Dose	-369.061	662.357	-0.557	0.578
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	-257.125	662.357	-0.388	0.698
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	-2779.600	662.357	-4.197	0.000

Table 2: Type II ANOVA for JO2 ~ Substrate * Dose

	Term	Sum Sq	Df	F value	Pr(>F)
Substrate	Substrate	501456649	5	84.879	0
Dose	Dose	1654342978	1	1400.103	0
Substrate:Dose	Substrate:Dose	362539320	5	61.365	0
Residuals	Residuals	387560330	328	NA	NA

Table 3: Type III ANOVA for JO2 ~ Genotype * Substrate * Dose

	Term	Sum Sq	Df	F value	Pr(>F)
(Intercept)	(Intercept)	276876709	1	392.648	0.000
Genotype	Genotype	3352741	1	4.755	0.030
Substrate	Substrate	170806542	5	48.445	0.000
Dose	Dose	248030403	1	351.740	0.000
Genotype:Substrate	Genotype:Substrate	8560933	5	2.428	0.035
Genotype:Dose	Genotype:Dose	3085222	1	4.375	0.037
Substrate:Dose	Substrate:Dose	162166791	5	45.995	0.000
Genotype:Substrate:Dose	Genotype:Substrate:Dose	8399558	5	2.382	0.038
Residuals	Residuals	222828039	316	NA	NA

Table 4: Mixed model fixed effects (lmer)

	Term	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	(Intercept)	143278.423	1231.723	308.850	301.478	0.000
GenotypeTg	GenotypeTg	22297.316	6437.103	308.850	3.464	0.001
SubstrateOcM (Octanoyl-Carnitine/Malate)	SubstrateOcM (Octanoyl-Carnitine/Malate)	-109528.826	6734.228	305.904	-16.265	0.000
SubstratePcM (Palmitoyl-Carnitine/Malate)	SubstratePcM (Palmitoyl-Carnitine/Malate)	-113298.516	6734.228	305.904	-16.824	0.000
SubstratePM (Pyruvate/Malate)	SubstratePM (Pyruvate/Malate)	-1342.392	6420.793	305.904	-0.209	0.835
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	-8026.608	6420.793	305.904	-1.250	0.212
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	-54350.934	6420.793	305.904	-8.465	0.000
Dose	Dose	9596.474	321.289	305.902	29.869	0.000
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate)	GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate)	-17511.829	9523.628	305.903	-1.839	0.067

	Term	Estimate	Std. Error	df	t value	Pr(>|t|)
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate)	GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate)	-10158.270	9523.628	305.903	-1.067	0.287
GenotypeTg:SubstratePM (Pyruvate/Malate)	GenotypeTg:SubstratePM (Pyruvate/Malate)	-11623.308	9080.373	305.903	-1.280	0.201
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine)	7909.048	9080.373	305.903	0.871	0.384
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	26976.291	9080.373	305.903	2.971	0.003
GenotypeTg:Dose	GenotypeTg:Dose	1513.622	454.371	305.903	3.331	0.001
SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	-7485.712	476.548	305.903	-15.708	0.000
SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	-7737.110	476.548	305.903	-16.236	0.000
SubstratePM (Pyruvate/Malate):Dose	SubstratePM (Pyruvate/Malate):Dose	26.091	454.371	305.904	0.057	0.954
SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	-532.464	454.371	305.904	-1.172	0.242
SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	-3706.611	454.371	305.904	-8.158	0.000
GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	GenotypeTg:SubstrateOcM (Octanoyl-Carnitine/Malate):Dose	-1278.135	673.941	305.903	-1.897	0.059
GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	GenotypeTg:SubstratePcM (Palmitoyl-Carnitine/Malate):Dose	-784.418	673.941	305.903	-1.164	0.245
GenotypeTg:SubstratePM (Pyruvate/Malate):Dose	GenotypeTg:SubstratePM (Pyruvate/Malate):Dose	-790.304	642.578	305.903	-1.230	0.220
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl-Carnitine):Dose	550.678	642.578	305.903	0.857	0.392
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl-Carnitine):Dose	1854.028	642.578	305.903	2.885	0.004

Table 5: Model fit statistics

Model	AIC	BIC	logLik	DF_Residual	N
lm_marginal	6383.189	6394.676	-3188.594	338	340
lm_raw	5732.667	5782.443	-2853.333	328	340
lm_multi	5568.486	5664.210	-2759.243	316	340
lmm	5002.741	5102.293	-2475.370	NA	340

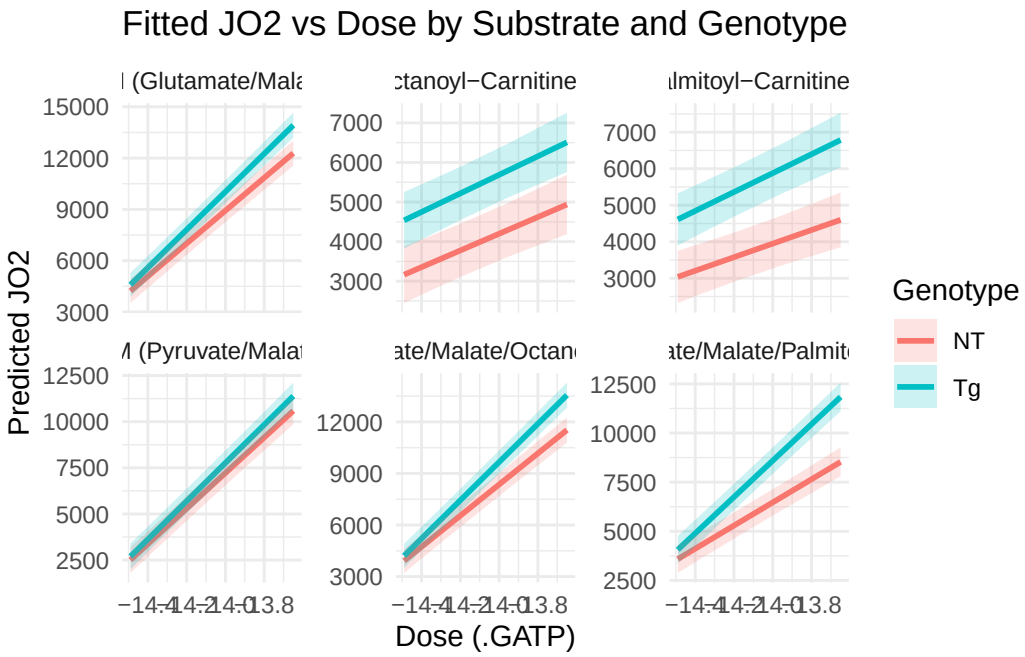

Figure 8: Predicted JO2 vs dose by substrate and genotype from mixed model

Table 6: Estimated genotype differences (Tg – NT) by substrate and dose

Substrate	Dose	Estimate	SE	df	t	p
GM (Glutamate/Malate)	-14.128	912.865	477.733	11.470	1.911	0.081
OcM (Octanoyl-Carnitine/Malate)	-14.128	1458.532	482.546	11.934	3.023	0.011
PcM (Palmitoyl-Carnitine/Malate)	-14.128	1836.859	482.546	11.934	3.807	0.003
PM (Pyruvate/Malate)	-14.128	454.965	477.733	11.470	0.952	0.361
PMOc (Pyruvate/Malate/Octanoyl-Carnitine)	-14.128	1041.937	477.733	11.470	2.181	0.051
PMPc (Pyruvate/Malate/Palmitoyl-Carnitine)	-14.128	1695.440	477.733	11.470	3.549	0.004

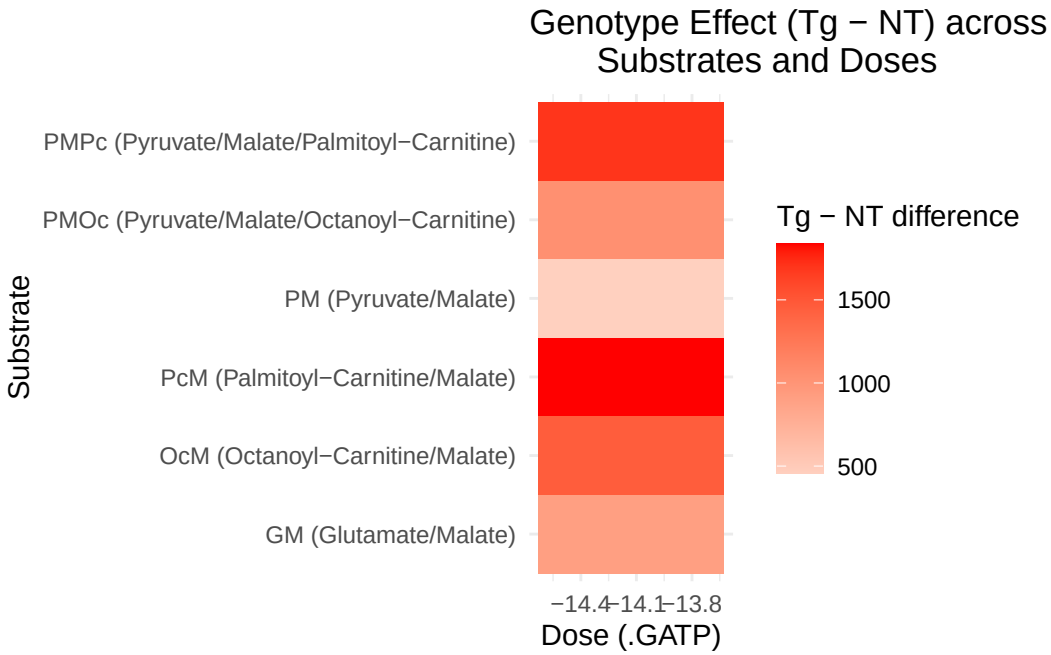

Figure 9: Genotype effect (Tg – NT) across substrates and doses

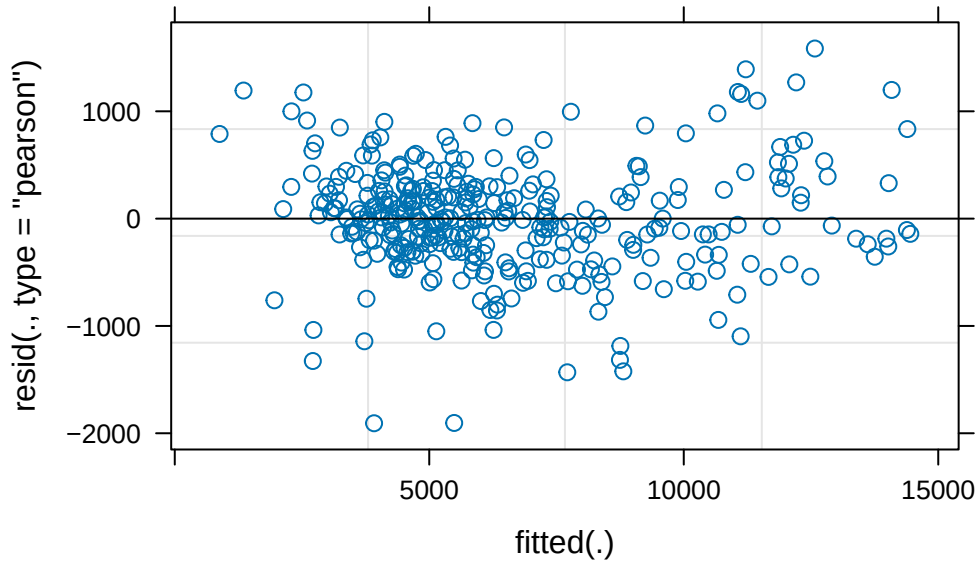

Figure 10: Residuals versus fitted values for mixed model

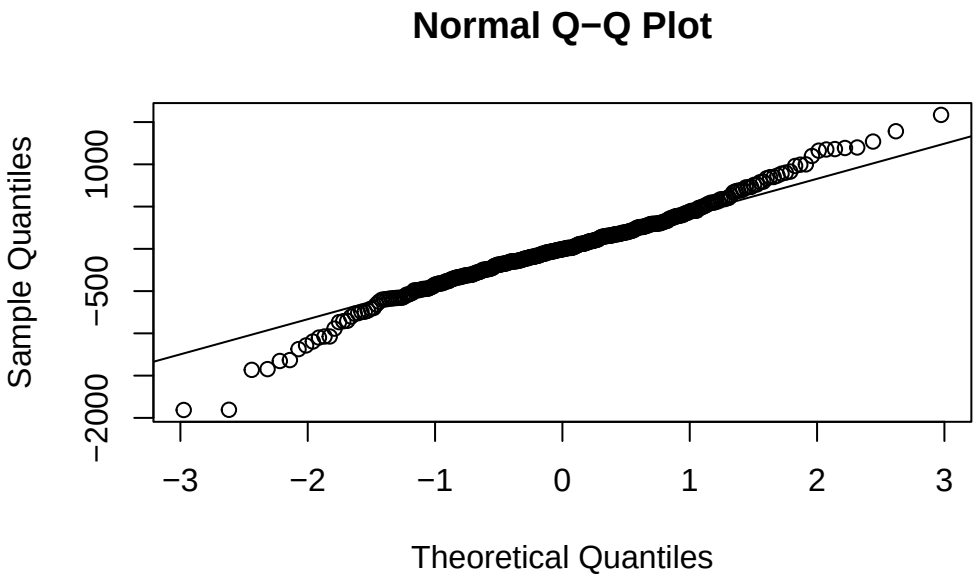

Figure 11: QQ plot of mixed model residuals