

STA 440 Case 2

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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 (JO_2), 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 ($\text{lm}(\text{JO2} \sim \text{Substrate} * \text{Dose})$)

This model considers only substrate and dose effects, ignoring genotype. It serves as a sanity check to establish whether fuel type and workload influence JO₂ as expected. Its main advantage is simplicity and interpretability, but it does not address the central research question concerning genotype differences.

Genotype-only model ($\text{lm}(\text{JO2} \sim \text{Genotype})$)

This specification provides a crude comparison of JO₂ 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 ($\text{lm}(\text{JO2} \sim \text{Genotype} * \text{Substrate} * \text{Dose})$)

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; $\text{lmer}(\text{JO2} \sim \text{Genotype} * \text{Substrate} * \text{Dose} + (1 \mid \text{Subject}))$)

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

Results

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.~
```

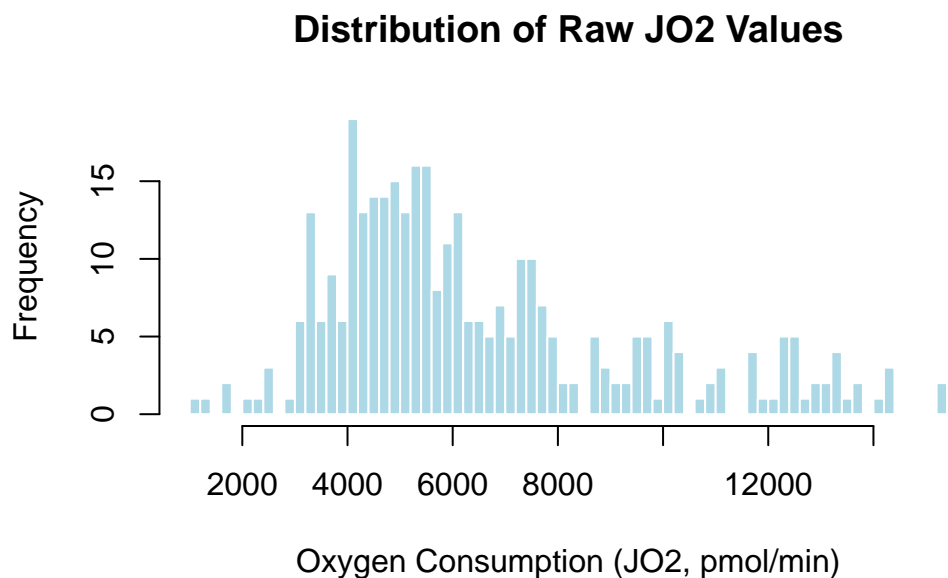


Figure 1: Distribution of raw JO2 values

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

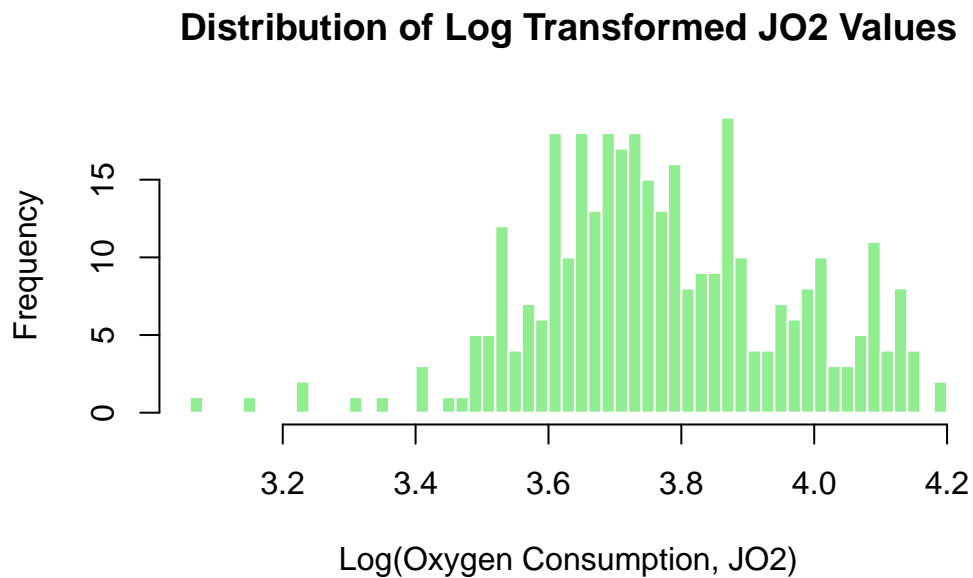


Figure 2: Distribution of log10-transformed JO2 values

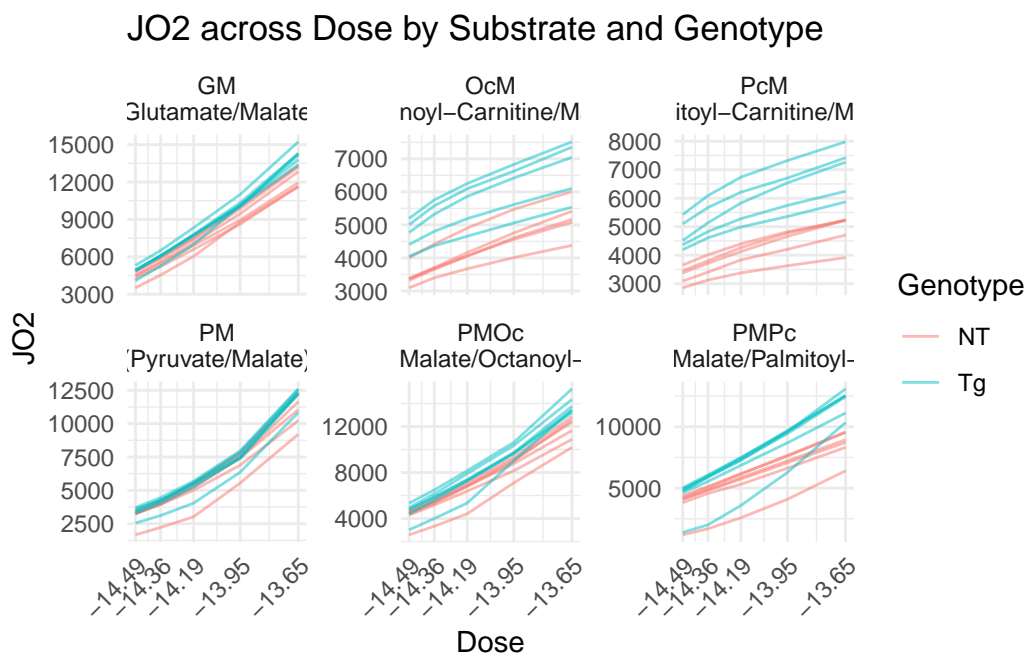


Figure 3: JO2 across dose by substrate and genotype

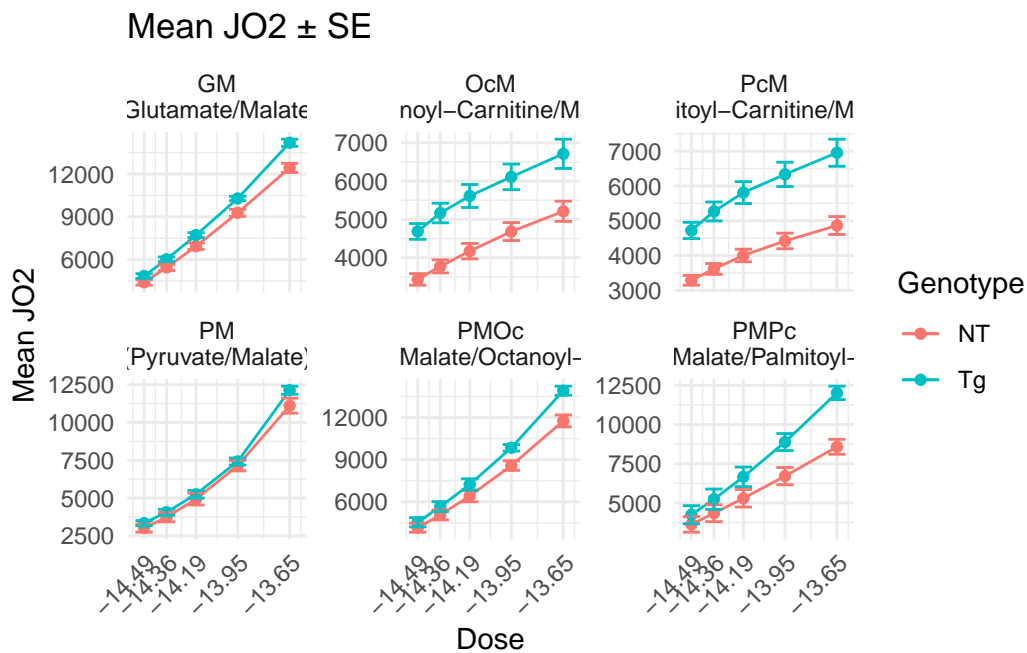


Figure 4: Mean JO2 with \pm SE by genotype and substrate

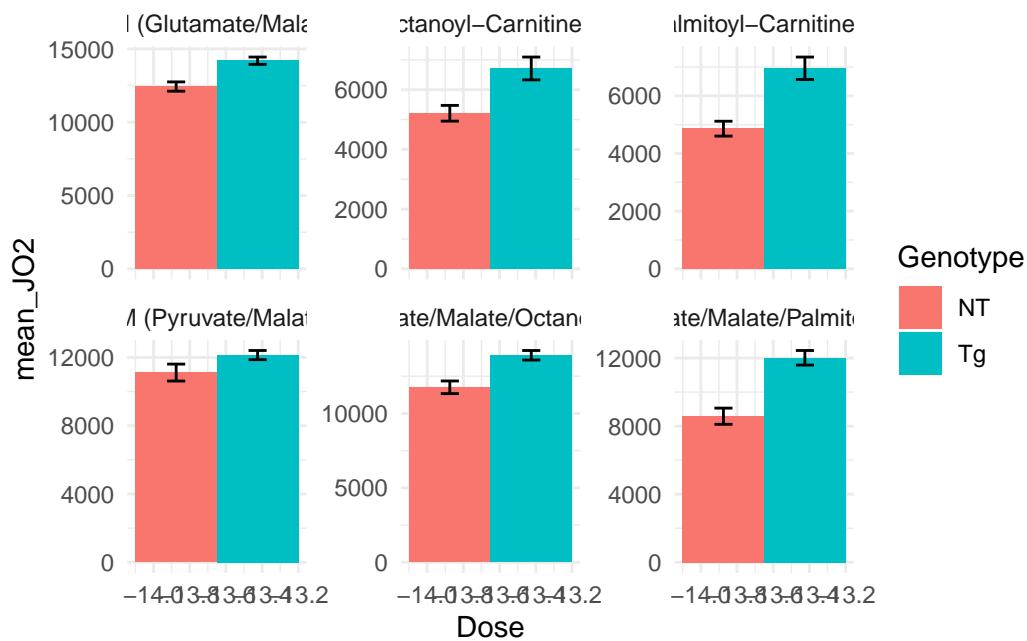


Figure 5: Mean JO2 at Basal and low dose (-13.65)

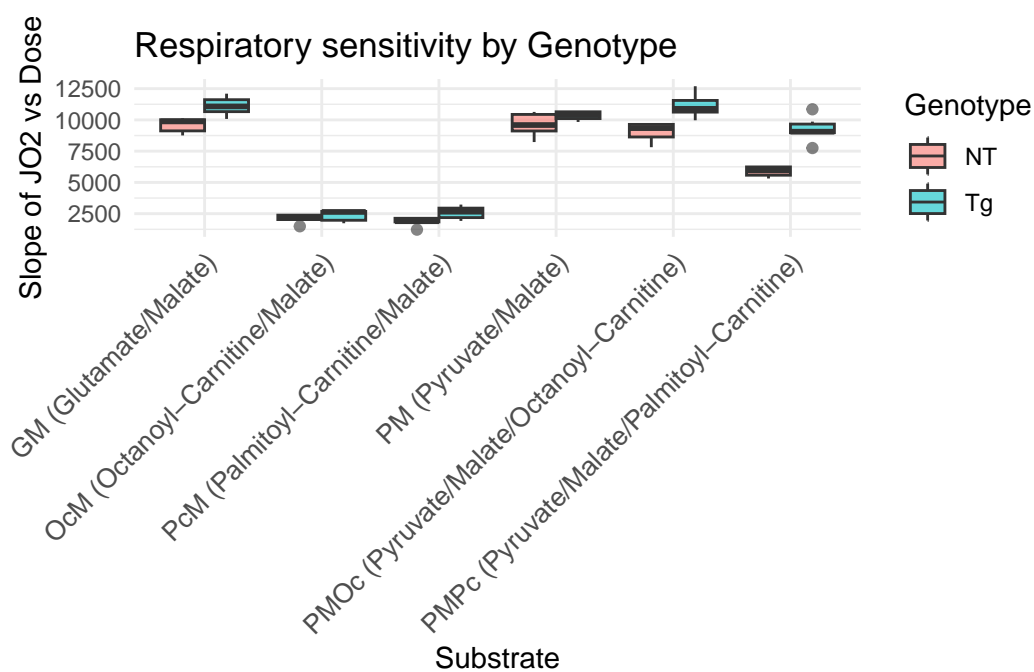


Figure 6: Distribution of JO2-dose slopes by genotype and substrate

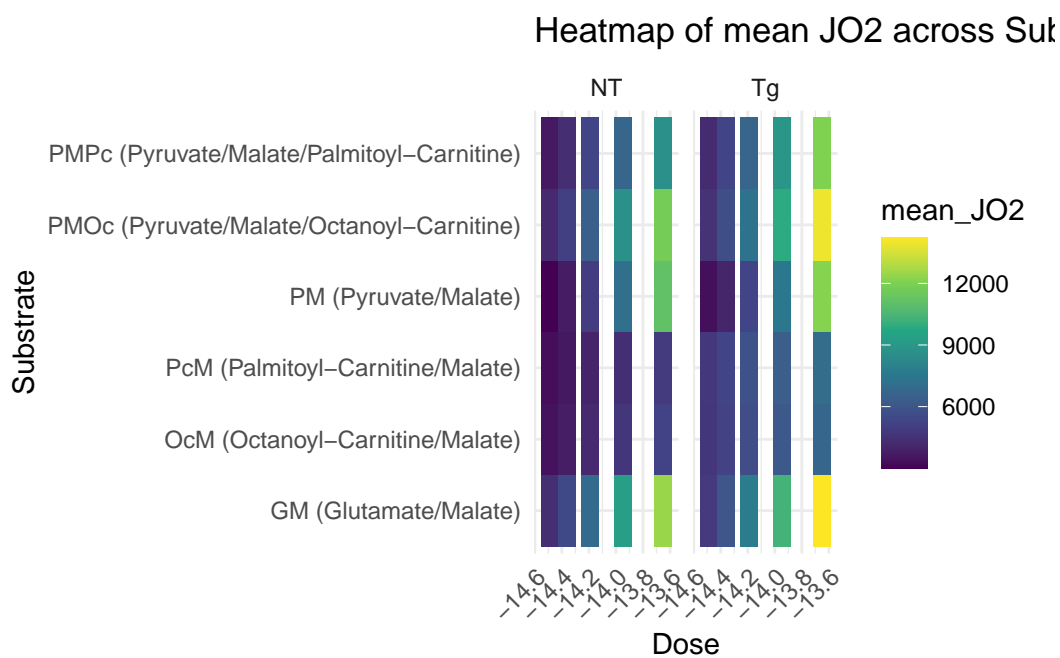


Figure 7: Heatmap of mean JO2 across substrates and doses

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: J02

	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: J02

	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: J02

	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 = J02 ~ 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: J02

	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: J02

	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: J02

	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: J02 ~ 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

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	t value	Pr(> t)
(Intercept)	(Intercept)	143278.423	11.723	12188.853	0.000
GenotypeTg	GenotypeTg	22297.366	437.103	50.853	0.001
SubstrateOcM	SubstrateOcM	- 6734.222	205.904	- 32.69	0.000
(Octanoyl-Carnitine/Malate)	(Octanoyl-Carnitine/Malate)	109528.826		16.265	
SubstratePcM (Palmitoyl-Carnitine/Malate)	SubstratePcM (Palmitoyl-Carnitine/Malate)	- 6734.222	205.904	- 32.69	0.000
SubstratePM	SubstratePM	113298.516		16.824	
(Pyruvate/Malate)	(Pyruvate/Malate)	- 6420.793	205.904	- 31.22	0.835
SubstratePMOc	SubstratePMOc	1342.392		0.209	
(Pyruvate/Malate/Octanoyl-Carnitine)	(Pyruvate/Malate/Octanoyl-Carnitine)	- 6420.793	205.904	- 31.22	0.212
SubstratePMPc	SubstratePMPc	8026.608		1.250	
(Pyruvate/Malate/Palmitoyl-Carnitine)	(Pyruvate/Malate/Palmitoyl-Carnitine)	- 6420.793	205.904	- 31.22	0.000
Dose	Dose	54350.934		8.465	
GenotypeTg:SubstrateOcM	GenotypeTg:SubstrateOcM	9596.474	21.289	450.902	0.000
(Octanoyl-Carnitine/Malate)	(Octanoyl-Carnitine/Malate)	- 9523.623	205.903	- 46.29	0.067
		17511.829		1.839	

		Std. Er- Estimate	t df	Pr(> t)
Term	Term			
GenotypeTg:SubstratePcM (Palmitoyl- Carnitine/Malate)	GenotypeTg:SubstratePcM (Palmitoyl- Carnitine/Malate)	- 9523.62305.903 - 10158.270	1.067	0.287
GenotypeTg:SubstratePM (Pyruvate/Malate)	GenotypeTg:SubstratePM (Pyruvate/Malate)	- 9080.37305.903 - 11623.308	1.280	0.201
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl- Carnitine)	GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl- Carnitine)	7909.049080.37305.903 13871	0.384	
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl- Carnitine)	GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl- Carnitine)	26976.29080.37305.903 2971	0.003	
GenotypeTg:Dose	GenotypeTg:Dose	1513.62254.371305.903 3331	0.001	
SubstrateOcM (Octanoyl- Carnitine/Malate):Dose	SubstrateOcM (Octanoyl- Carnitine/Malate):Dose	- 476.548305.903 - 7485.712	15.708	0.000
SubstratePcM (Palmitoyl- Carnitine/Malate):Dose	SubstratePcM (Palmitoyl- Carnitine/Malate):Dose	- 476.548305.903 - 7737.110	16.236	0.000
SubstratePM (Pyruvate/Malate):Dose	SubstratePM (Pyruvate/Malate):Dose	26.091 454.371305.903 1057	0.954	
SubstratePMOc (Pyruvate/Malate/Octanoyl- Carnitine):Dose	SubstratePMOc (Pyruvate/Malate/Octanoyl- Carnitine):Dose	- 454.371305.904 - 532.464	1.172	0.242
SubstratePMPc (Pyruvate/Malate/Palmitoyl- Carnitine):Dose	SubstratePMPc (Pyruvate/Malate/Palmitoyl- Carnitine):Dose	- 454.371305.904 - 3706.611	8.158	0.000
GenotypeTg:SubstrateOcM (Octanoyl- Carnitine/Malate):Dose	GenotypeTg:SubstrateOcM (Octanoyl- Carnitine/Malate):Dose	- 673.941305.903 - 1278.135	1.897	0.059
GenotypeTg:SubstratePcM (Palmitoyl- Carnitine/Malate):Dose	GenotypeTg:SubstratePcM (Palmitoyl- Carnitine/Malate):Dose	- 673.941305.903 - 784.418	1.164	0.245
GenotypeTg:SubstratePM (Pyruvate/Malate):Dose	GenotypeTg:SubstratePM (Pyruvate/Malate):Dose	- 642.578305.903 - 790.304	1.230	0.220
GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl- Carnitine):Dose	GenotypeTg:SubstratePMOc (Pyruvate/Malate/Octanoyl- Carnitine):Dose	550.678642.578305.903 3857	0.392	
GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl- Carnitine):Dose	GenotypeTg:SubstratePMPc (Pyruvate/Malate/Palmitoyl- Carnitine):Dose	1854.02342.578305.903 2885	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

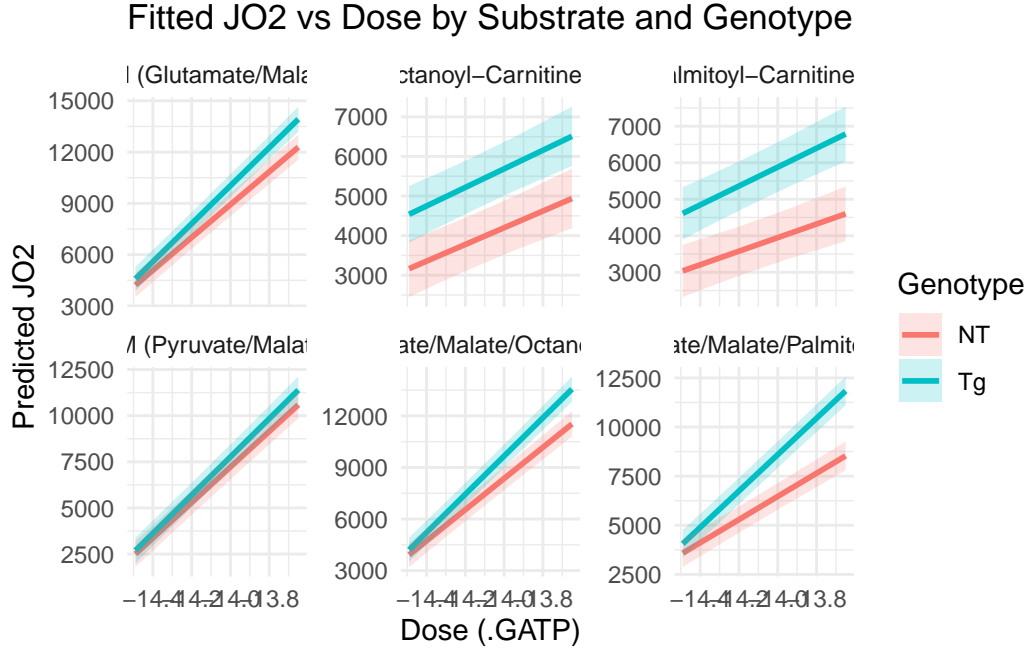


Figure 8: Predicted JO2 vs dose by substrate and genotype from mixed model

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	-14.128	1041.937	477.733	11.470	2.181	0.051
(Pyruvate/Malate/Octanoyl-Carnitine)						
PMPc	-14.128	1695.440	477.733	11.470	3.549	0.004
(Pyruvate/Malate/Palmitoyl-Carnitine)						

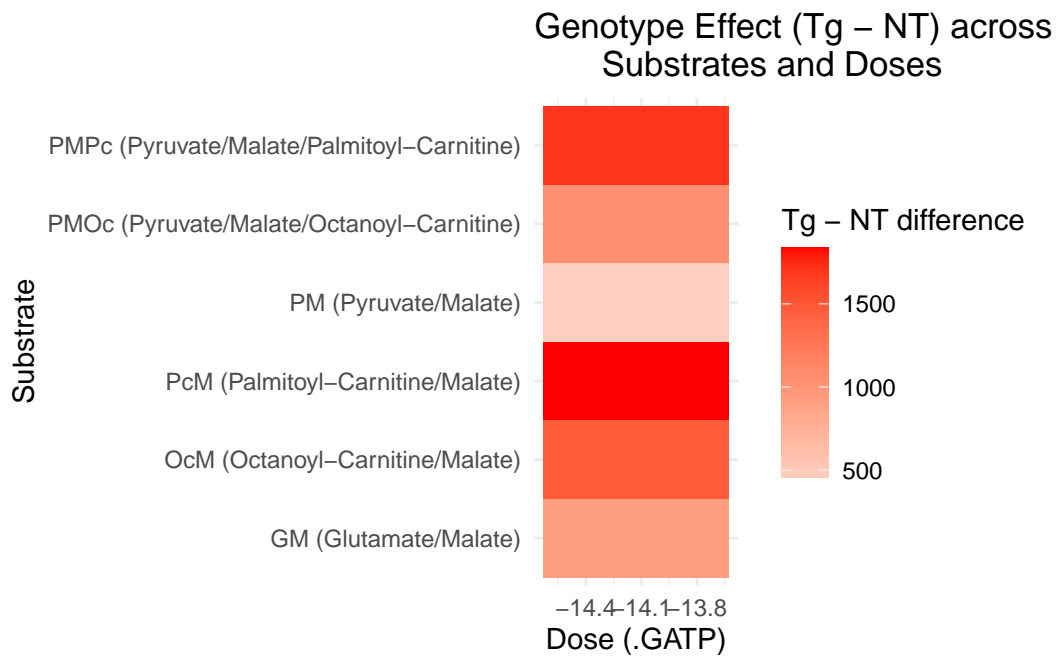


Figure 9: Genotype effect (Tg - NT) across substrates and doses

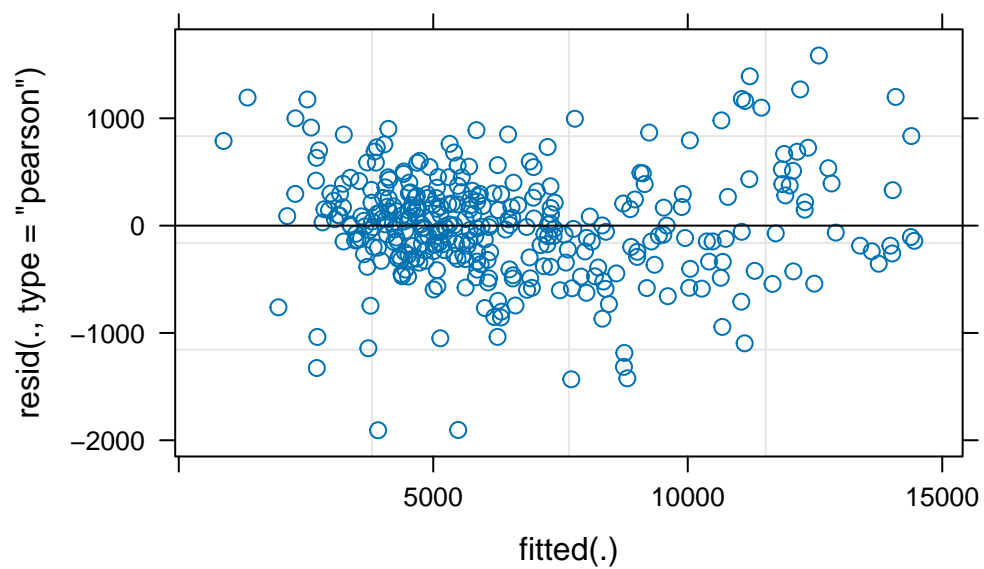


Figure 10: Residuals versus fitted values for mixed model

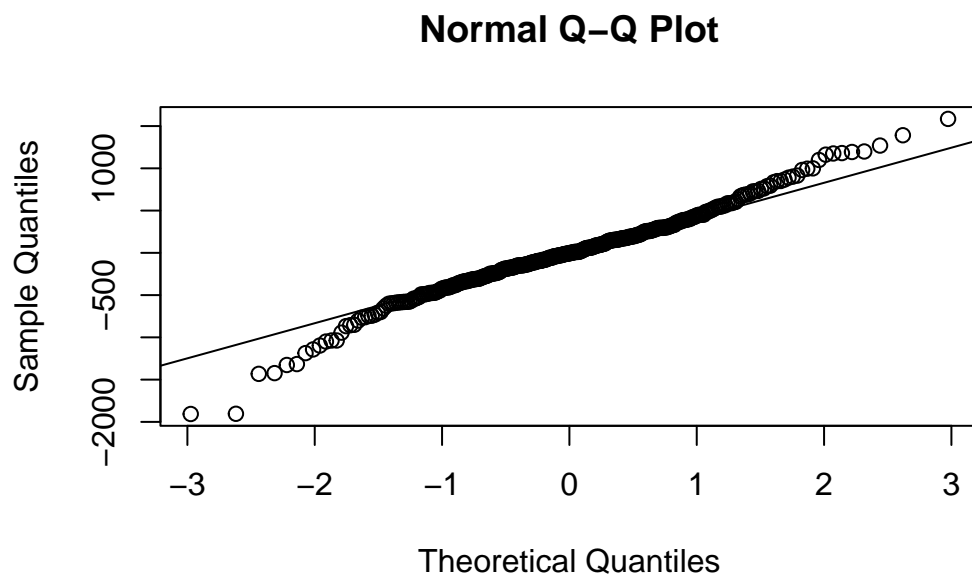


Figure 11: QQ plot of mixed model residuals