Exploration of Genotype on the Metabolic Efficiency of the Mitochondria

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1 Background

The mitochondria are considered the "powerhouse" of the cell, responsible for generating the cell's usable energy. Their proper function is critical for overall health, as disruptions to their function are associated with various health issues, such as cancer, heart disease, and Alzheimer's.

One way to examine mitochondrial function is by using the multiplexed assay platform, a laboratory method that allows researchers to measure multiple dimensions of mitochondrial activity across different substrates and energy demand conditions. By measuring respiration rates under different combinations of substrates across different experimental settings, such as genetic background and dose, researchers hope to better understand these effects on the metabolic and functional phenotypes of mitochondria.

The main motivation for our analysis is to quantitatively test hypotheses about genetic changes on mitochondrial efficiency and energy production, and whether there is evidence that genotype effects (transgenic vs. natural mice) depend on substrate and/or dose. By building a modeling framework, we hope to determine how mitochondrial efficiency varies by substrate, genotype, and dose while capturing both fixed and random sources of variation.

2 Exploratory Data Analysis

The data for the study were collected from skeletal muscle mitochondria isolated from non-transgenic (natural type) and transgenic mice. The independent variables used from the dataset, that were measured to assess whether systematic differences exist in VO_2 production, are the treatment variable *genotype* (whether the mouse is transgenic or not), *substrate type* (the substrate provided to mitochondria), and *dose* (estimated levels of free energy to ATP hydrolysis).

Looking at Figure *1*, genotype appears to affect VO_2 production. Across nearly all substrates, transgenic mice display higher VO_2 production than natural mice. For substrates of $PMOc$

and $PMPc$, we can see different slopes for VO_2 production vs. dose, and as the doses become higher, the effects of the genotype become more significant. This suggests a need for an interaction between dose and substrate. The effect is most pronounced when the doses are higher in $PMPc$ and $PMOc$, and for OcM and PcM we see a clear higher VO_2 production for all doses. Additionally, Substrates involving Octanoyl Carnitine (Oc) and Palmitoyl-Carnitine (Pc) have a more pronounced separation between transgenic vs natural mice.

OcM and PcM show a relatively flat dose-response curve for both genotypes, which suggests limited sensitivity to dose changes but the $PMOc$ and $PMPc$ substrates highlight a stronger genotype effect dependent on dose. This points to an interaction between substrate and genotype where certain substrates amplify the genotype-specific differences in the VO_2 production efficiency.

Pair - Level Variation

The researchers' experimental design, which matched a transgenic mouse with a natural type mouse and tested each pair on a different day, could induce some added variation that dose and substrate cannot account for. This is because the experimental setup could vary slightly day-to-day, influencing the measurement of our response variable VO_2.

Looking at Figures *2* and *3*, we can see that there does in fact seem to be systematic differences on the pair level. Pair 5 exhibits a much larger gap between VO_2 production of transgenic and natural type mice for OcM and PcM compared to pair 2. We saw enough variation across every pair to warrant consideration in our final modeling decisions.

Substrate vs. Amino Acid Modeling

In addition to visual exploration, we also compared two different ways of representing substrate effects in preliminary linear models.

1. **Amino Acid Model:** Breaks down substrates into their biochemical building blocks (glutamate, pyruvate, etc), assuming these act independently and additively.
2. **Substrate Model:** Treats the substrate bundles (GM, OcM, etc) as its own categorical condition.

From Table *1*, we can see that the substrate model provided the better fit based on model comparison statistics when comparing two basic additive models (AIC = 7386.29, adj. $R^2 = 0.755$) compared to the amino acid model (AIC = 7439.92, adj. $R^2 = 0.721$), explaining the variation in VO_2 more effectively. The amino acid model is limited in that it assumes the effects of these fuels are additive and constant, which is unlikely biologically. The representation is still useful in understanding which fuel components may be driving observed differences.

3 Modeling

To account for all of the points noted above, we chose to fit a fully interactive linear model regressing genotype, dose, and substrate on VO_2. Furthermore, we included a random intercept for pair, allowing us to include this added variation in the model while retaining the ability to predict on an unobserved pair. Finally, we treated dose as a numerical value because the researchers' assumption of linearity between dose and VO2, and removed the -12.95 dose observation because that was also suggested by researchers.

From Table *2*, we can see a log likelihood value of -2489.7 for our chosen model. Compared to a model excluding genotype, this likelihood is statistically significant as a more preferred model according to a chi-squared test. Furthermore, our models intraclass correlation (ICC), which is the ratio of pair variability to total variability, is high. An ICC value of 0.644 is a strong piece of evidence in support of including a random intercept for pair, as it signifies that the variation across pairs is non-neglible.

Finally, we can see the conditional R^2 value, which represents the variance explained by our model. By allowing each pair have its own intercept we explain about 96.5% of the total variability in our data, higher than the 90% explained marginally by only the fixed effects.

$$VO_2 = Genotype * Dose * Substrate + (1|pair) + \epsilon$$

To check the adequacy of our model, we plotted a quantile-quantile plot of the residuals (Figure *4*). As seen on the graph, the residuals fall closely along the 45 degree reference line with minor deviations at the tails, indicating the assumption of normally distributed errors is reasonably satisfied.

4 Results

Research question 1: Is there a genetic difference?

To assess evidence of an overall difference between transgenic and natural type mice, we conducted a t-test between the groups, accounting for the known relationships between substrates and dose we saw in our EDA. Table *2* shows the difference in means between transgenic and natural type mice, as well as a t-statistic and p-value. We see that transgenic mice, on average, have a VO2 production 1192.72 units higher than natural type mice, controlling for dose and substrate. So, we can reject the null hypothesis that there is no difference between the two groups.

Research question 2: How does our model explain the dependence of the relationship on Dose, Substrate, and Pair?

As seen in Figure *5* our fitted model provides strong evidence of genetic association with mitochondrial efficiency across several substrate-dose combinations, where transgenic mice demonstrate a steeper increase in oxygen consumption as dose increases. For example, under the PMPc substrate, the estimated dose-response slope had a significant difference between genotypes- for every 0.1 unit increase in dose, VO_2 is predicted to increase by 926 units in transgenic mice compared to only 589 units in natural mice.

However, these effects were not uniform across all conditions. When observing substrate PcM, we see very different predicted values for the lowest dose level (4580 for transgenic compared to 3063 for natural type) that are significantly different. The slopes of the dose-response are more similar than for PMPC similar, with an increase of 258 units in VO2 for transgenic mice compared to 185 for natural type. This suggests that genotype has a baseline effect on mitochondrial efficiency under PcM but is not as strongly dependent on dose.

Together, these results demonstrate that mitochondrial efficiency is shaped by the interactions between genotype, substrate, and dose, with the magnitude of the effect of genotype depending on the substrate provided and the energy demand.

5 Conclusion and Future Work

Our analysis showed strong evidence that genotype significantly influences VO_2 production conditional on both substrate and dose. Across nearly all experimental conditions, transgenic mice displayed a higher VO_2 production relative to natural mice, with some substrates amplifying this effect more than others. These findings show that the transgenic genotype is associated with enhanced metabolic efficiency.

However, one major limitation can be seen in Figure *6*, the residual plot across dosage levels. We see some evidence of a nonlinear relationship between dose and VO_2 production conditional on substrate and genotype, something that was not accounted for in our model. We chose to leave the relationship linear because it was our understanding that the researchers' had some biological motivation behind this claim. Since our analysis shows evidence arguing against this claim, further work should include interrogating these assumptions, especially among higher dosage levels.

In addition, future work should focus on exploring a broader range of substrates and leveraging larger samples to better account for variability across experimental pairs. These extensions would help clarify the extent to which the observed genotype effects generalize across different biological and experimental contexts.

6 Appendix

Exploratory Data Analysis

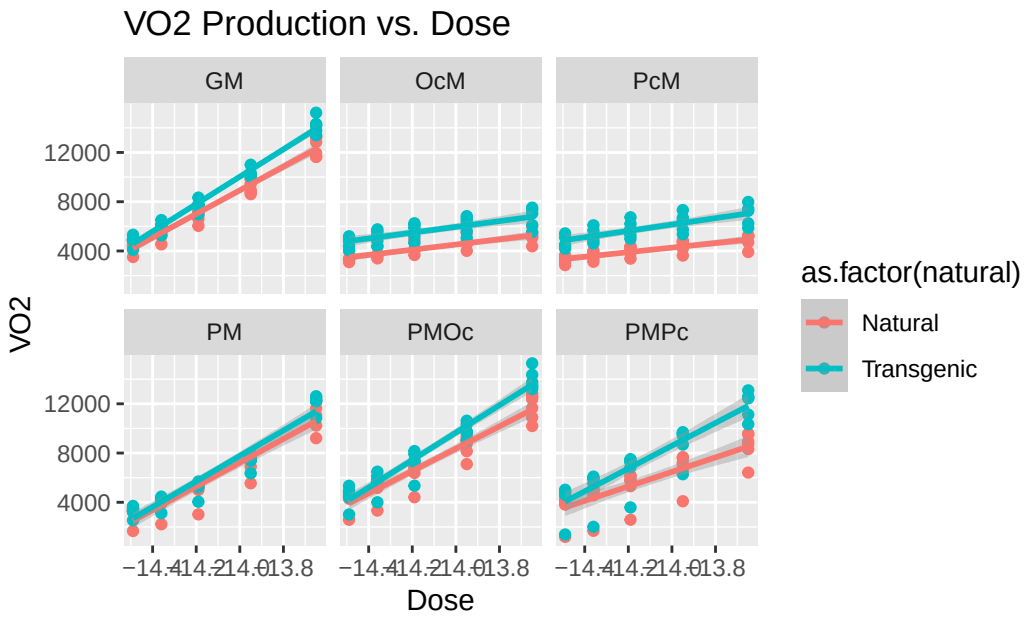

Figure 1

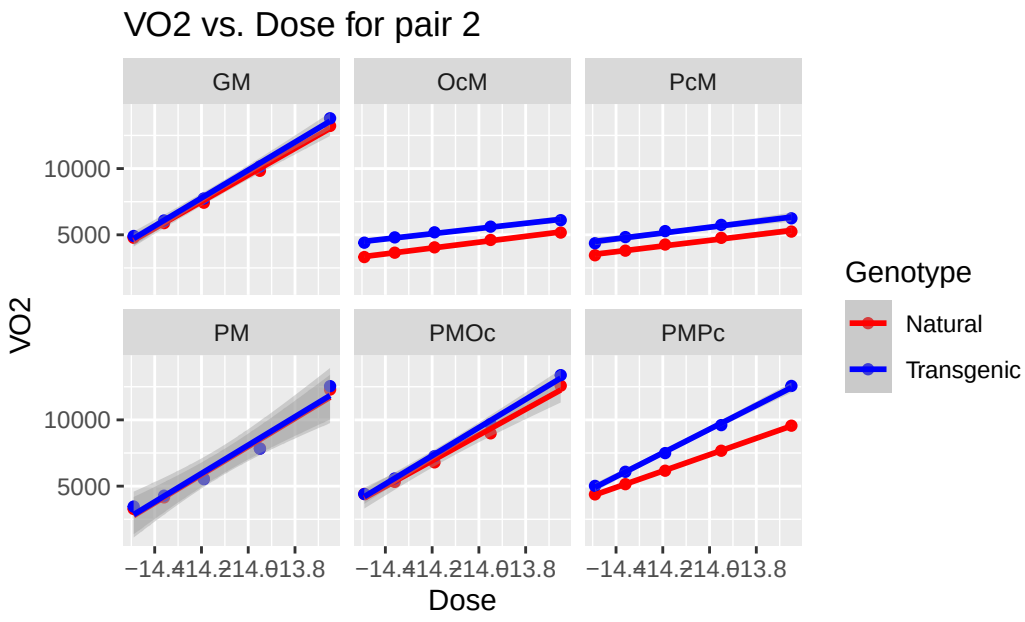

Figure 2

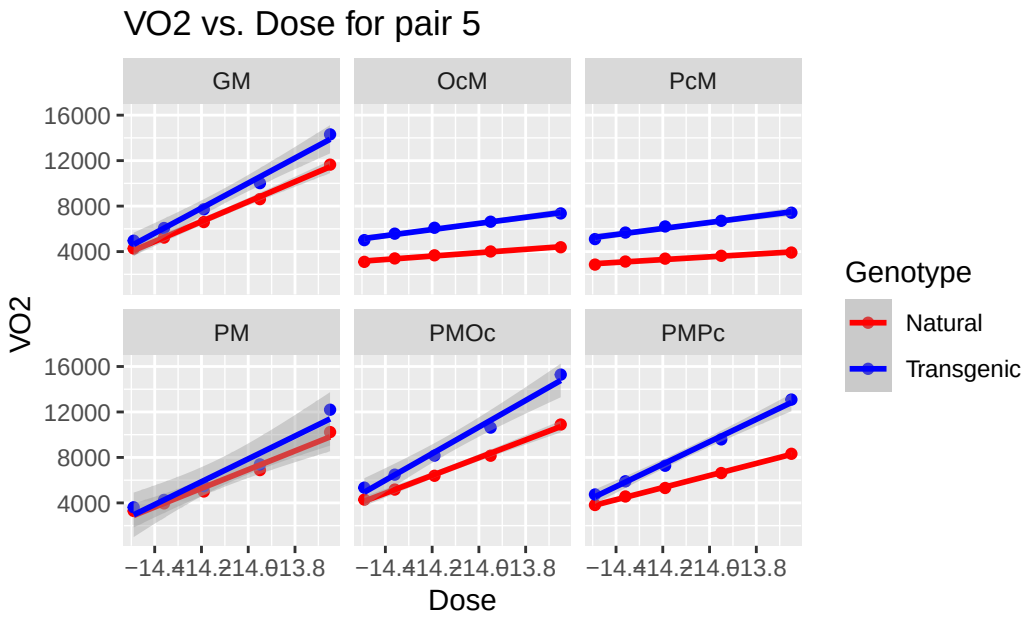
VO2 vs. Dose for pair 5
GM
OcM
PcM
PM
PMOc
PMPc
16000
12000
8000
4000
VO2
−14.4
14.2
14.0
13.8
Dose
Genotype
Natural
Transgenic

Figure 3

Table 1: Model comparison using AIC and Adjusted R^2

Model	df	AIC	Adjusted_R2
Amino Acids	8	7439.921	0.721
Substrate	9	7386.290	0.755

Modeling

Table 2: Model performance metrics

Measure	Value
LogLik	-2489.7
ICC	0.644
Conditional_R2	0.965
Marginal_R2	0.901

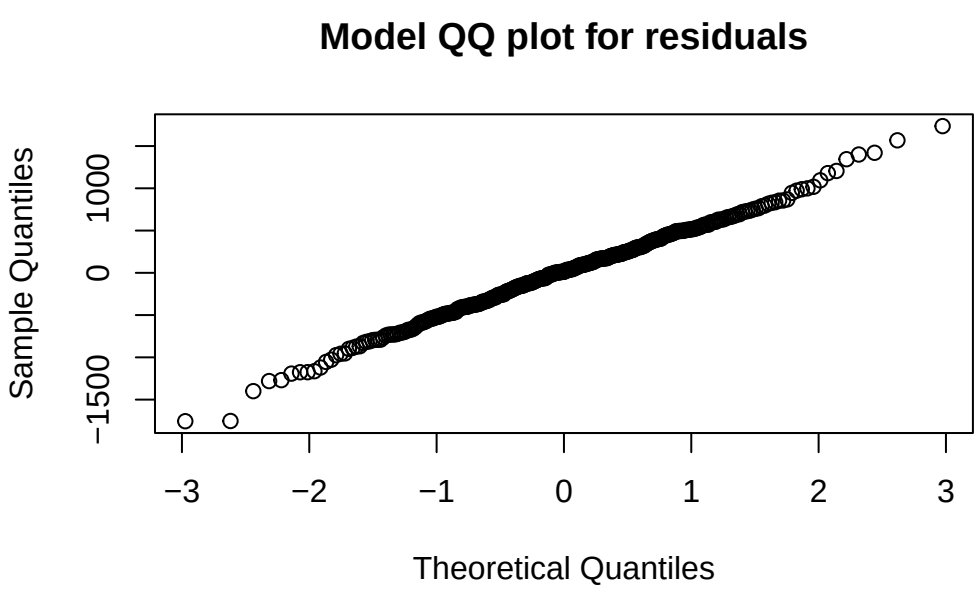

Figure 4

Results

Table 3: Overall effect of genotype

Measure	Value
MeanDifference	1192.2
TValue	12.177
PValue	0

Table 4: Model coefficient estimates and P-values

	Term	P_value
(Intercept)	(Intercept)	0.0000000
naturalTransgenic	naturalTransgenic	0.0014019
Dose	Dose	0.0000000
SubstrateOcM	SubstrateOcM	0.0000000
SubstratePcM	SubstratePcM	0.0000000
SubstratePM	SubstratePM	0.8462603
SubstratePMOc	SubstratePMOc	0.2468113
SubstratePMPc	SubstratePMPc	0.0000000
naturalTransgenic:Dose	naturalTransgenic:Dose	0.0021683
naturalTransgenic:SubstrateOcM	naturalTransgenic:SubstrateOcM	0.0878677
naturalTransgenic:SubstratePcM	naturalTransgenic:SubstratePcM	0.3202895
naturalTransgenic:SubstratePM	naturalTransgenic:SubstratePM	0.2356952
naturalTransgenic:SubstratePMOc	naturalTransgenic:SubstratePMOc	0.4194504
naturalTransgenic:SubstratePMPc	naturalTransgenic:SubstratePMPc	0.0061697
Dose:SubstrateOcM	Dose:SubstrateOcM	0.0000000
Dose:SubstratePcM	Dose:SubstratePcM	0.0000000
Dose:SubstratePM	Dose:SubstratePM	0.9575291
Dose:SubstratePMOc	Dose:SubstratePMOc	0.2775669
Dose:SubstratePMPc	Dose:SubstratePMPc	0.0000000
naturalTransgenic:Dose:SubstrateOcM	naturalTransgenic:Dose:SubstrateOcM	0.0793397
naturalTransgenic:Dose:SubstratePcM	naturalTransgenic:Dose:SubstratePcM	0.2808306
naturalTransgenic:Dose:SubstratePM	naturalTransgenic:Dose:SubstratePM	0.2545133
naturalTransgenic:Dose:SubstratePMOc	naturalTransgenic:Dose:SubstratePMOc	0.4269678
naturalTransgenic:Dose:SubstratePMPc	naturalTransgenic:Dose:SubstratePMPc	0.0077983

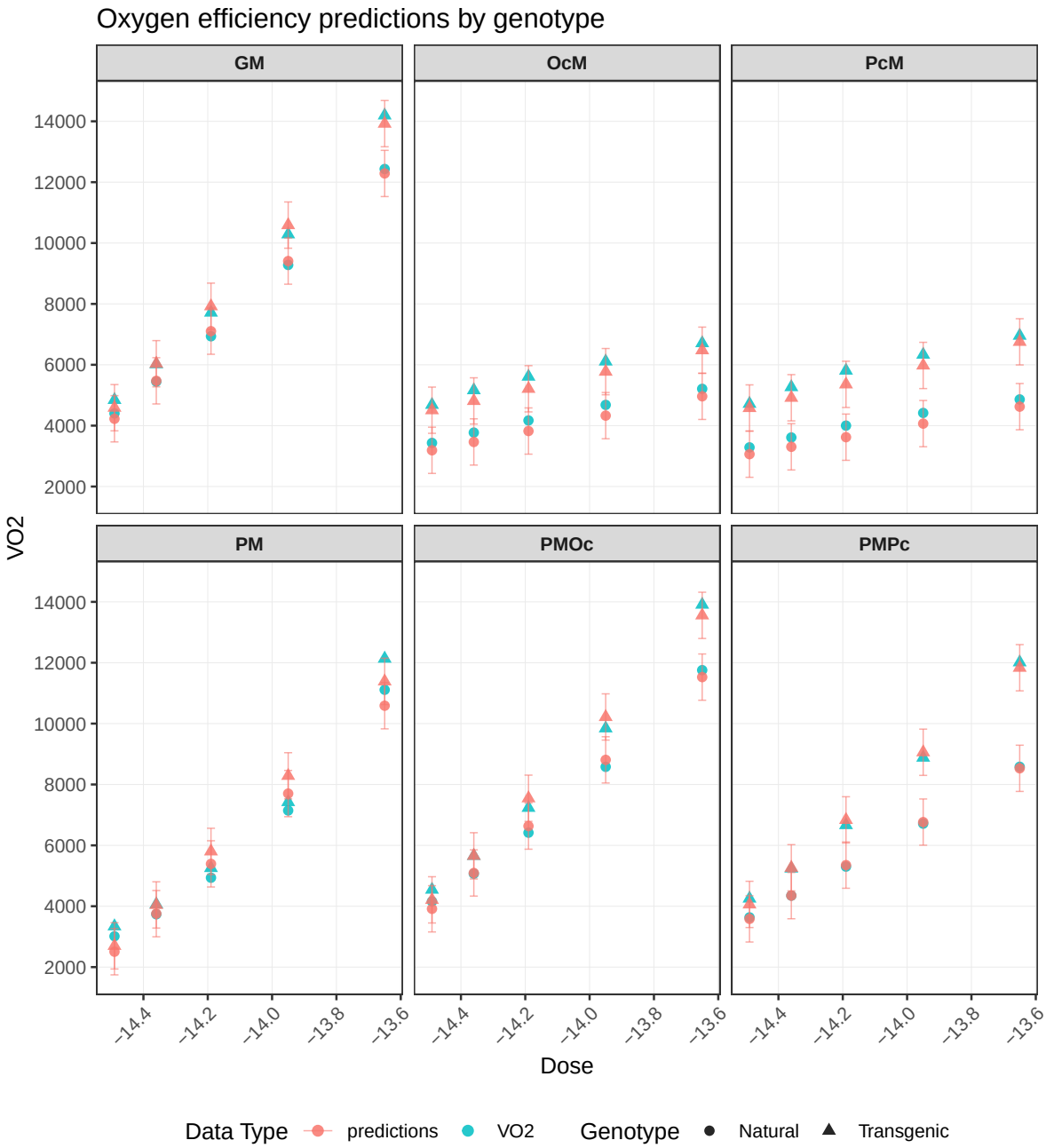
Oxygen efficiency predictions by genotype
GM
OcM
PcM
PM
PMOc
PMPc
VO2
14000
12000
10000
8000
6000
4000
2000
−14.4
−14.2
−14.0
−13.8
−13.6
Dose
Data Type
predictions
VO2
Genotype
Natural
Transgenic

Figure 5

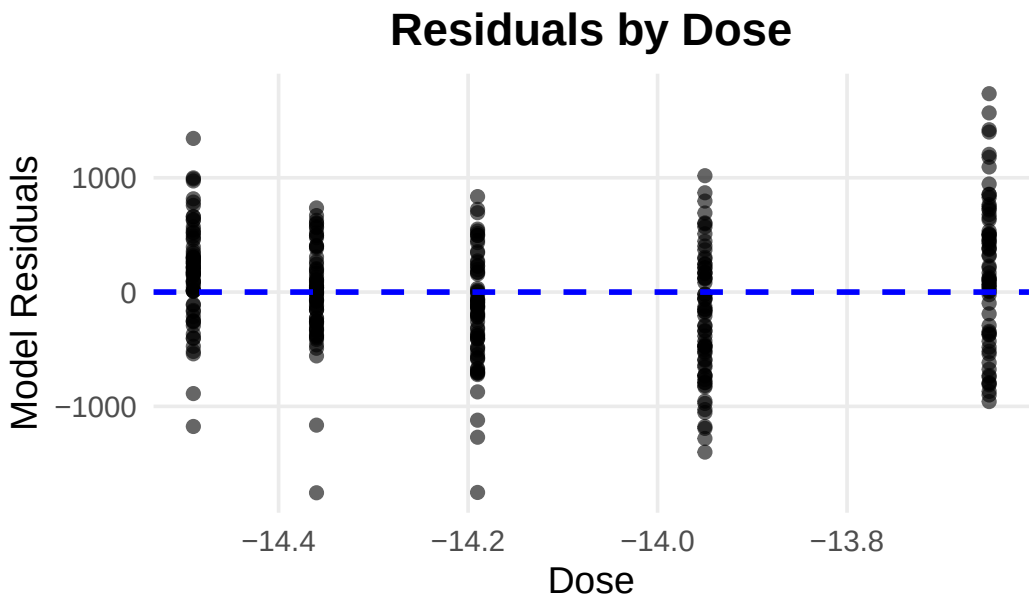
Residuals by Dose
Model Residuals
1000
0
−1000
−14.4
−14.2
−14.0
−13.8
Dose

Figure 6