Exploration of Multiple Treatments 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 through oxidative phosphorylation, a process necessary for all biological processes, particularly in high-demand organs such as the heart, brain, and muscles. 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 (control) and transgenic mice. The independent variables used from the dataset were measured to assess whether systematic differences exist in

$$VO_2$$

production, our response variable, is 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, we can see that genotype affects VO_2 production. Across nearly all substrates, we can see that the 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. We can also see that substrates involving Octanoyl Carnitine Oc and Palmitoyl-Carnitine Pc has 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 and $PMOc$ and $PMPc$ substrates highlight a stronger genotype effect as the transgenic mice has a more pronounced effect to 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 Figure 2, we can see that there does in fact seem to be systematic differences on the pair level. For example, pair 5 exhibits a much larger gap between VO_2 production of transgenic and natural type mice for OcM and PcM. Across every pair, we see enough variation between the genotype-VO_2 relationship 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 (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. However, the amino acid model is limited in that it assumes the effects of these fuels are additive and constant, which is unlikely biologically, given the complicated interactions of the mitochondria. The representation is still useful in understanding which fuel components may be driving observed differences.

Conclusions

1. Genotype appears significantly correlated with VO_2 production: transgenic mice had higher VO_2 than natural mice across most conditions
2. Substrate and dose seem to matter as well: We saw certain substrates had a larger effect in the VO_2 production as $PMOc$ and $PMPc$ tended to affect the transgenic mice more while OcM and PcM tended to have an equal effect on transgenic and natural mice. This relationship was highly dependent on dose.
3. While researchers do not need to know the specific measurement predictions for each pair, we need to account for variation on the pair level in our model.
4. Treating substrates as bundled conditions seems to capture variation in VO_2 more effectively.

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.

From Table 1, 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. 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.

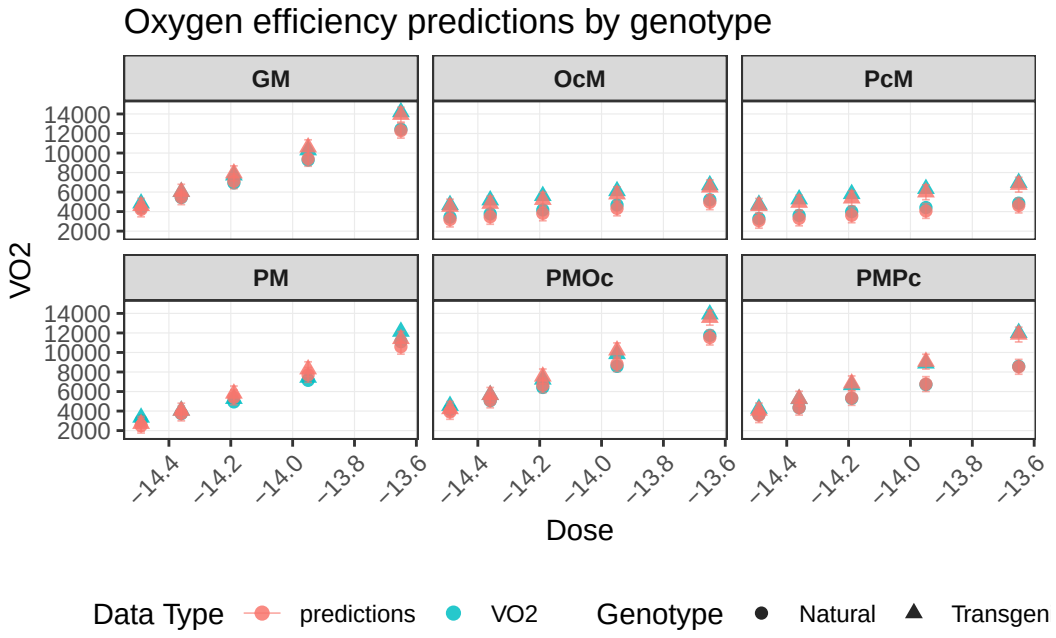
Finally, we see that the conditional R^2 value in Table 2 represents the variance explained by our model. By allowing each pair have its own intercept we explain about 96.5% of the total variability in our data, higher than the 90% explained marginally by the fixed effects.

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

$$Model\ 2: \ VO_2 = Dose * Substrate + (1|pair) + \epsilon$$

4 Analysis

TODO: bonferroni correction for p-values, include/analyze graph and give interpretation examples (see presentation).



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 transgenic geotype is associated with enhanced metabolic efficiency

However, one major limitation can be seen in Figure 4, 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 live 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.

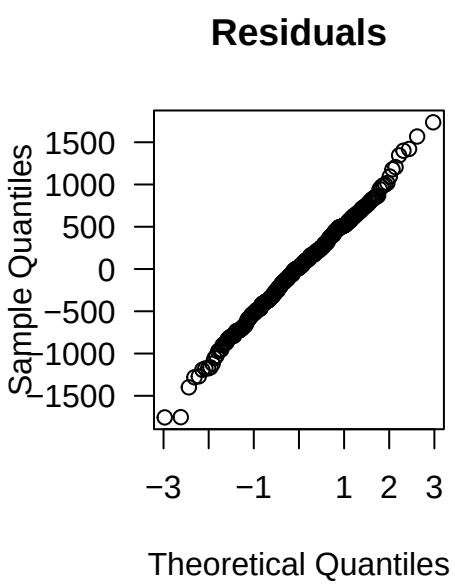
6 Appendix

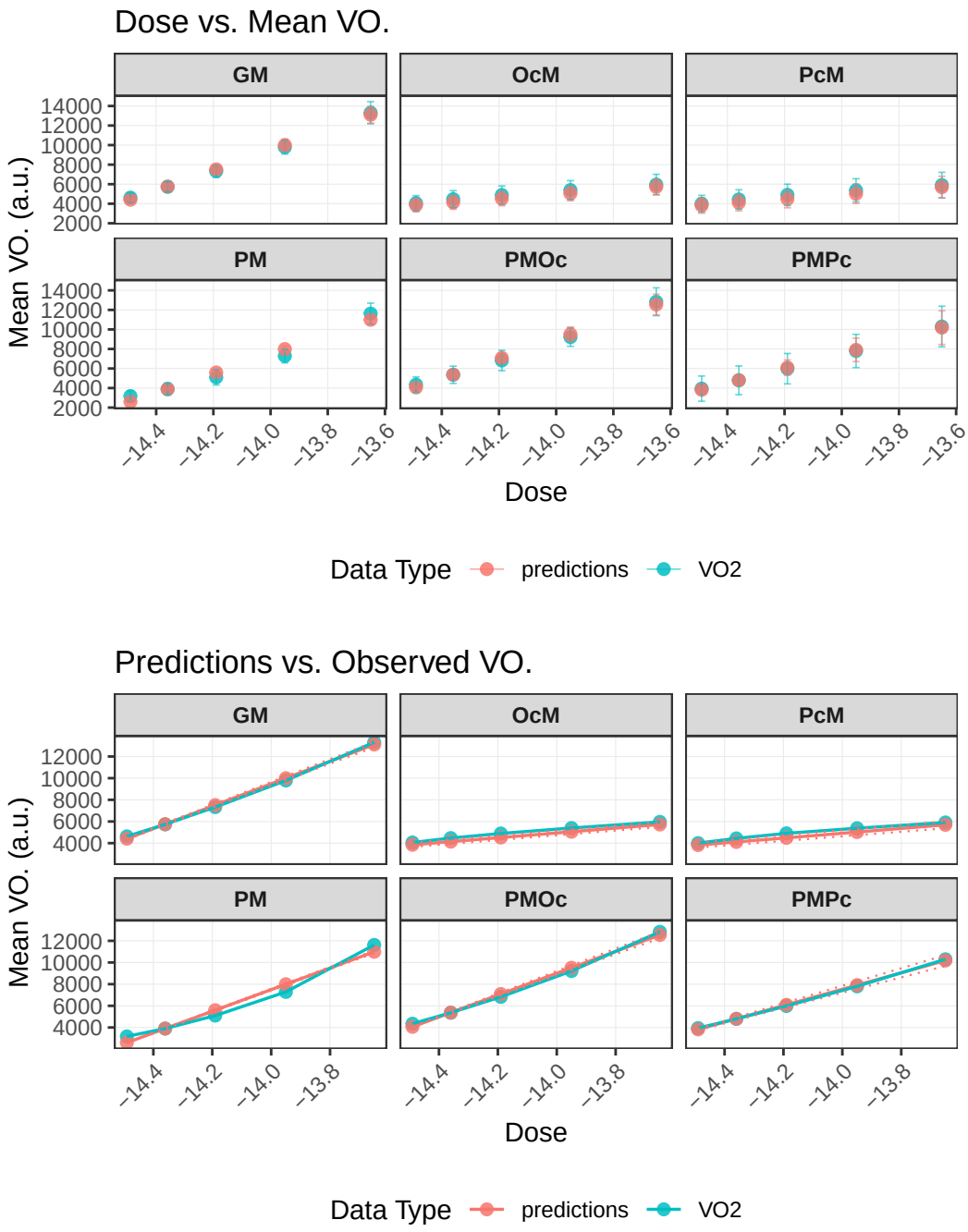
Exploratory Data Analysis

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




Dose vs. Mean VO.
GM
OcM
PcM
PM
PMOc
PMPc
Mean VO. (a.u.)
14000
12000
10000
8000
6000
4000
2000
−14.4
−14.2
−14.0
−13.8
−13.6
Dose
Data Type
predictions
VO2

Predictions vs. Observed VO.
GM
OcM
PcM
PM
PMOc
PMPc
Mean VO. (a.u.)
12000
10000
8000
6000
4000
−14.4
−14.2
−14.0
−13.8
Dose
Data Type
predictions
VO2