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 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 (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.

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.

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. 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, 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 X). 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

We fit the linear mixed-effects model shown above with VO_2 as the response variable. As seen in Figure , the 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, and the model revealed that the genotype effect depends on both substrate and dose. When observing substrate PcM, we can see visible differences between natural and transgenic mice across all dose levels as the difference starts off and continues to be statistically significant, as seen by the error bars. The slopes of the dose-response are similar for both transgenic and non-transgenic mice, with the transgenic group consistently having a higher intercept, suggesting that genotype has a baseline effect on mitochondrial efficiency under PcM but is not strongly dependent on dose.

However, for substrate PMPc, we can see the error bars between transgenic and non-transgenic mic overlapping more substantially at lower doses, but as the dose increases, the difference between mitochondrial efficiency for transgenic and natural mice becomes statistically significant, showing the effect of genotype is amplified with increasing dose for certain substrates. For OcM and PcM substrates, the slopes were relatively flatter, suggesting less sensitivity to dose changes compared to PMOc and PMPc dose-genotype interactions which became more sensitive at higher doses.

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 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

```
`geom_smooth()` using formula = 'y ~ x'
```

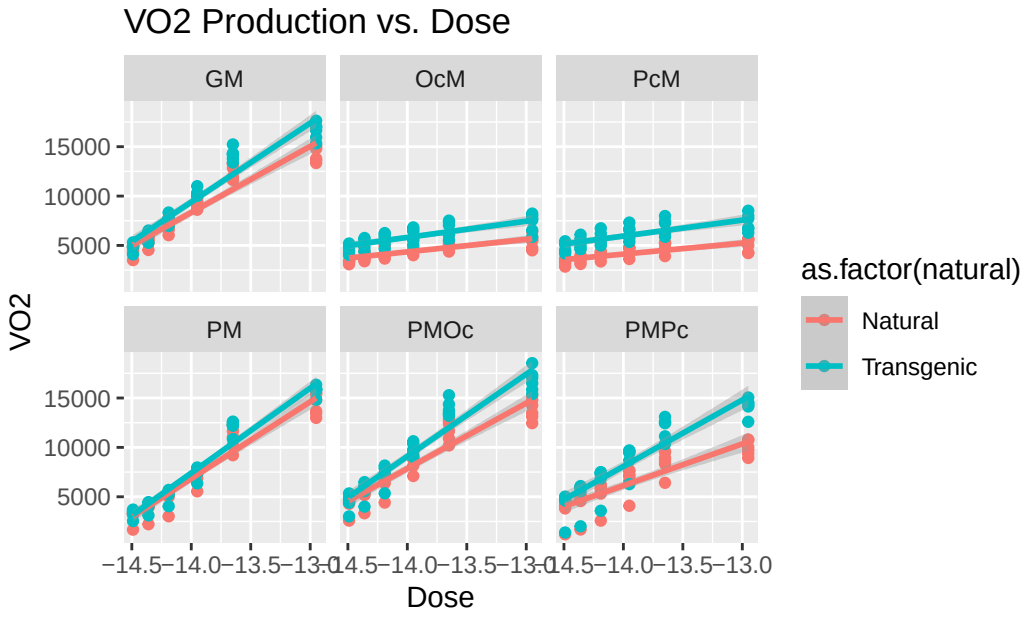
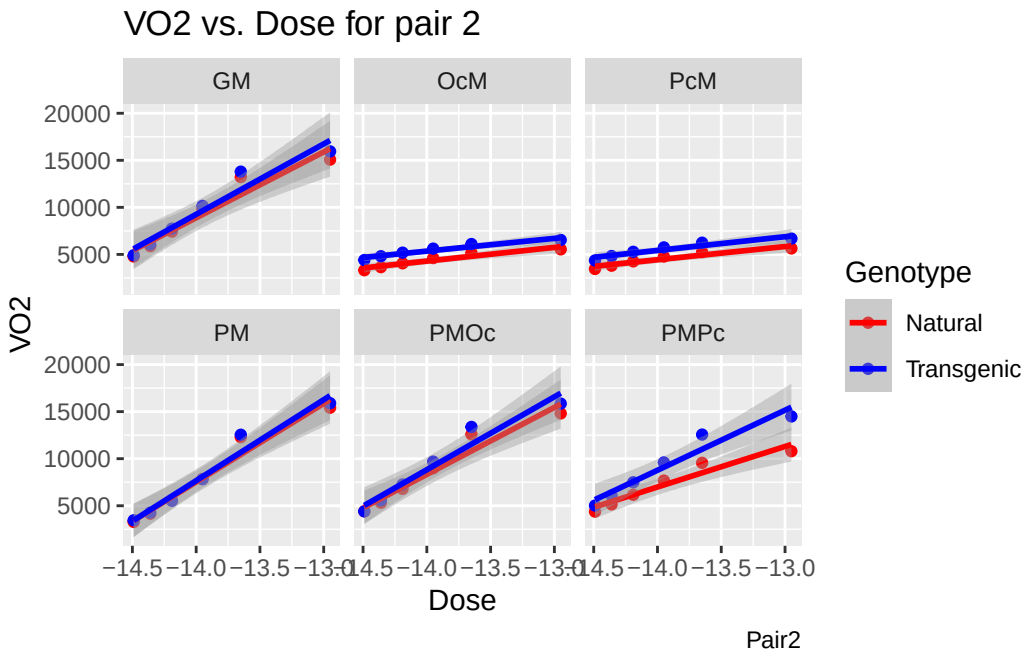


Figure 1



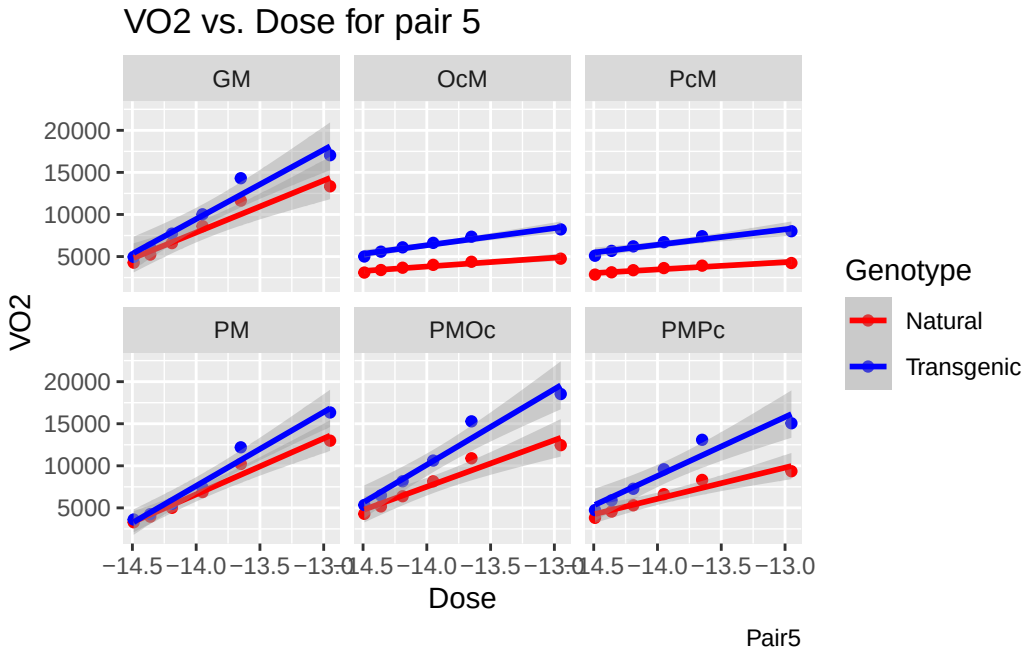


Table 1: Model comparison using AIC and Adjusted R^2

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

Results

Table 3: Overall effect of genotype

Measure	Value
MeanDifference	1192.2
TValue	12.177
PValue	0

Table 4: Table 3

	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

	Term	P_value
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

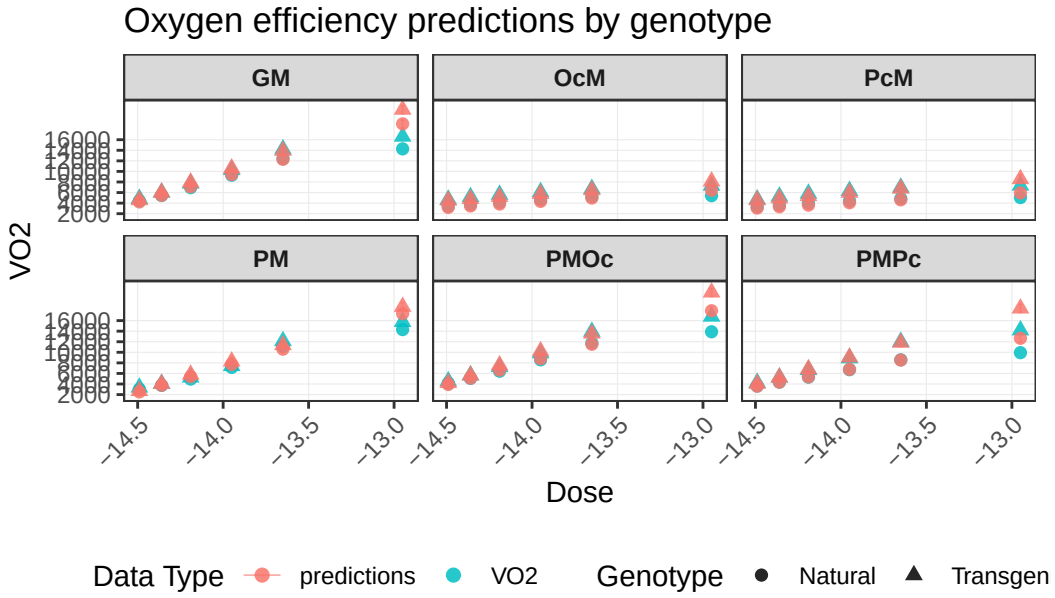


Figure 2

```
Call:
lm(formula = VO2 ~ Substrate:natural + Dose:natural:Substrate,
    data = data)

Residuals:
    Min      1Q  Median      3Q     Max
-3254.1  -370.8   186.2   536.4  1727.3
```

```
Coefficients: (1 not defined because of singularities)
                                    Estimate Std. Error t value Pr(>|t|)
(Intercept)                         138201.0     7230.7  19.113  < 2e-16 ***
SubstrateGM:naturalNatural            5077.4    10225.7   0.497 0.619863
SubstrateOcM:naturalNatural        -104126.4    10724.8  -9.709  < 2e-16 ***
SubstratePcM:naturalNatural        -107896.1    10724.8 -10.060  < 2e-16 ***
SubstratePM:naturalNatural            3735.0    10225.7   0.365 0.715163
SubstratePMOc:naturalNatural         -2949.2    10225.7  -0.288 0.773223
SubstratePMPc:naturalNatural        -49273.5    10225.7  -4.819 2.25e-06 ***
SubstrateGM:naturalTransgenic        27374.7    10225.7   2.677 0.007815 **
SubstrateOcM:naturalTransgenic      -99396.5    10724.8  -9.268  < 2e-16 ***
SubstratePcM:naturalTransgenic      -95812.6    10724.8  -8.934  < 2e-16 ***
SubstratePM:naturalTransgenic        14409.0    10225.7   1.409 0.159790
SubstratePMOc:naturalTransgenic      27257.2    10225.7   2.666 0.008081 **
SubstratePMPc:naturalTransgenic           NA         NA      NA       NA
SubstrateGM:naturalNatural:Dose       9596.5      511.7  18.755  < 2e-16 ***
SubstrateOcM:naturalNatural:Dose      2110.8      560.5   3.766 0.000198 ***
SubstratePcM:naturalNatural:Dose      1859.4      560.5   3.317 0.001015 **
SubstratePM:naturalNatural:Dose       9622.6      511.7  18.806  < 2e-16 ***
SubstratePMOc:naturalNatural:Dose     9064.0      511.7  17.714  < 2e-16 ***
SubstratePMPc:naturalNatural:Dose     5889.9      511.7  11.511  < 2e-16 ***
SubstrateGM:naturalTransgenic:Dose   11110.1      511.7  21.713  < 2e-16 ***
SubstrateOcM:naturalTransgenic:Dose   2346.2      560.5   4.186 3.69e-05 ***
SubstratePcM:naturalTransgenic:Dose   2588.6      560.5   4.618 5.64e-06 ***
SubstratePM:naturalTransgenic:Dose   10345.9      511.7  20.219  < 2e-16 ***
SubstratePMOc:naturalTransgenic:Dose 11128.3      511.7  21.748  < 2e-16 ***
SubstratePMPc:naturalTransgenic:Dose  9257.5      511.7  18.092  < 2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 839.7 on 316 degrees of freedom
  (20 observations deleted due to missingness)
Multiple R-squared:  0.9233,    Adjusted R-squared:  0.9177
F-statistic: 165.4 on 23 and 316 DF,  p-value: < 2.2e-16



Call:
lm(formula = VO2 ~ natural * Dose * Substrate, data = data)

Residuals:
    Min      1Q  Median      3Q     Max
-3254.1  -370.8   186.2   536.4  1727.3
```

```
Coefficients:
                                    Estimate Std. Error t value Pr(>|t|)
(Intercept)                       143278.42    7230.68  19.815  < 2e-16 ***
naturalTransgenic                  22297.32   10225.72   2.181   0.0300 *
Dose                                9596.47     511.68  18.755  < 2e-16 ***
SubstrateOcM                     -109203.79   10724.83 -10.182  < 2e-16 ***
SubstratePcM                     -112973.48   10724.83 -10.534  < 2e-16 ***
SubstratePM                        -1342.39   10225.72  -0.131   0.8956
SubstratePMOc                      -8026.61   10225.72  -0.785   0.4331
SubstratePMPc                     -54350.93   10225.72  -5.315 2.02e-07 ***
naturalTransgenic:Dose             1513.62     723.63   2.092   0.0373 *
naturalTransgenic:SubstrateOcM   -17567.40   15167.20  -1.158   0.2476
naturalTransgenic:SubstratePcM   -10213.84   15167.20  -0.673   0.5012
naturalTransgenic:SubstratePM    -11623.31   14461.35  -0.804   0.4221
naturalTransgenic:SubstratePMOc    7909.05   14461.35   0.547   0.5848
naturalTransgenic:SubstratePMPc   26976.20   14461.35   1.865   0.0631 .
Dose:SubstrateOcM                 -7485.71     758.95  -9.863  < 2e-16 ***
Dose:SubstratePcM                 -7737.11     758.95 -10.195  < 2e-16 ***
Dose:SubstratePM                     26.09     723.63   0.036   0.9713
Dose:SubstratePMOc                 -532.46     723.63  -0.736   0.4624
Dose:SubstratePMPc                -3706.61     723.63  -5.122 5.26e-07 ***
naturalTransgenic:Dose:SubstrateOcM -1278.14    1073.31  -1.191   0.2346
naturalTransgenic:Dose:SubstratePcM  -784.42    1073.31  -0.731   0.4654
naturalTransgenic:Dose:SubstratePM   -790.30    1023.37  -0.772   0.4405
naturalTransgenic:Dose:SubstratePMOc  550.68    1023.37   0.538   0.5909
naturalTransgenic:Dose:SubstratePMPc 1854.02    1023.37   1.812   0.0710 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 839.7 on 316 degrees of freedom
  (20 observations deleted due to missingness)
Multiple R-squared:  0.9233,    Adjusted R-squared:  0.9177
F-statistic: 165.4 on 23 and 316 DF,  p-value: < 2.2e-16



Call:
lm(formula = VO2 ~ Substrate - 1, data = data)

Residuals:
    Min      1Q  Median      3Q     Max
-5366.1 -1939.5  -445.7  1327.1  7567.6
```

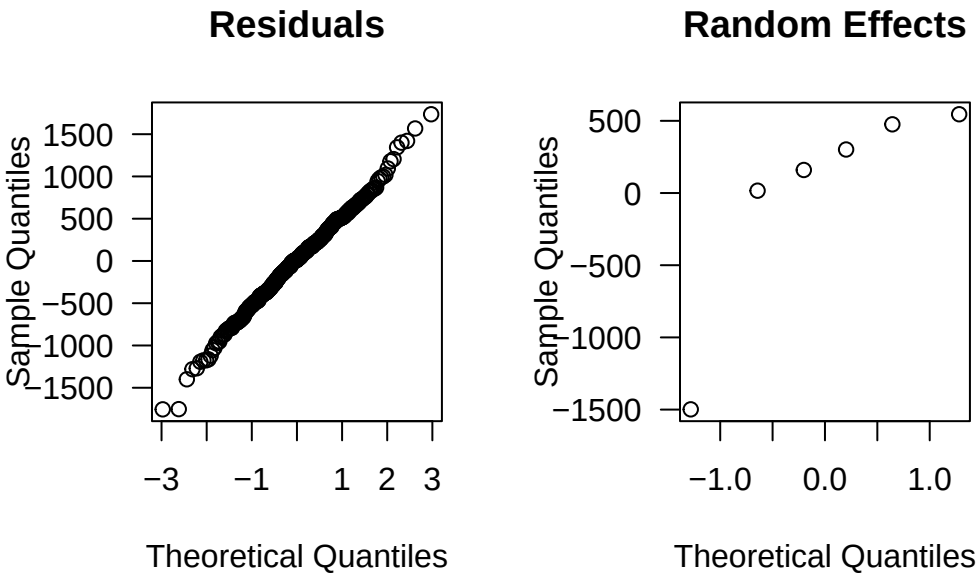
```
Coefficients:
              Estimate Std. Error t value Pr(>|t|)
SubstrateGM     8155.9      346.4   23.55   <2e-16 ***
SubstrateOcM    4955.3      379.4   13.06   <2e-16 ***
SubstratePcM    4926.5      379.4   12.98   <2e-16 ***
SubstratePM     6215.9      346.4   17.95   <2e-16 ***
SubstratePMOc   7716.4      346.4   22.28   <2e-16 ***
SubstratePMPc   6563.2      346.4   18.95   <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 2683 on 334 degrees of freedom
  (20 observations deleted due to missingness)
Multiple R-squared:  0.8611,    Adjusted R-squared:  0.8586
F-statistic: 345.1 on 6 and 334 DF,  p-value: < 2.2e-16


Linear mixed model fit by REML. t-tests use Satterthwaite's method [
lmerModLmerTest]
Formula: VO2 ~ natural * Dose * Substrate + (1 | pair)
   Data: data

REML criterion at convergence: 4979.4

Scaled residuals:
     Min       1Q   Median       3Q      Max
-3.09075 -0.64782  0.02403  0.63668  3.05598

Random effects:
 Groups   Name        Variance Std.Dev.
 pair     (Intercept) 582983   763.5
 Residual             322707   568.1
Number of obs: 340, groups:  pair, 6

Fixed effects:
                                    Estimate Std. Error         df t value
(Intercept)                       143278.42    4901.42     313.21  29.232
naturalTransgenic                  22297.32    6917.62     310.97   3.223
Dose                                9596.47     346.15     310.97  27.724
SubstrateOcM                     -109503.46    7255.28     310.97 -15.093
SubstratePcM                     -113273.15    7255.28     310.97 -15.613
SubstratePM                        -1342.39    6917.62     310.97  -0.194
```

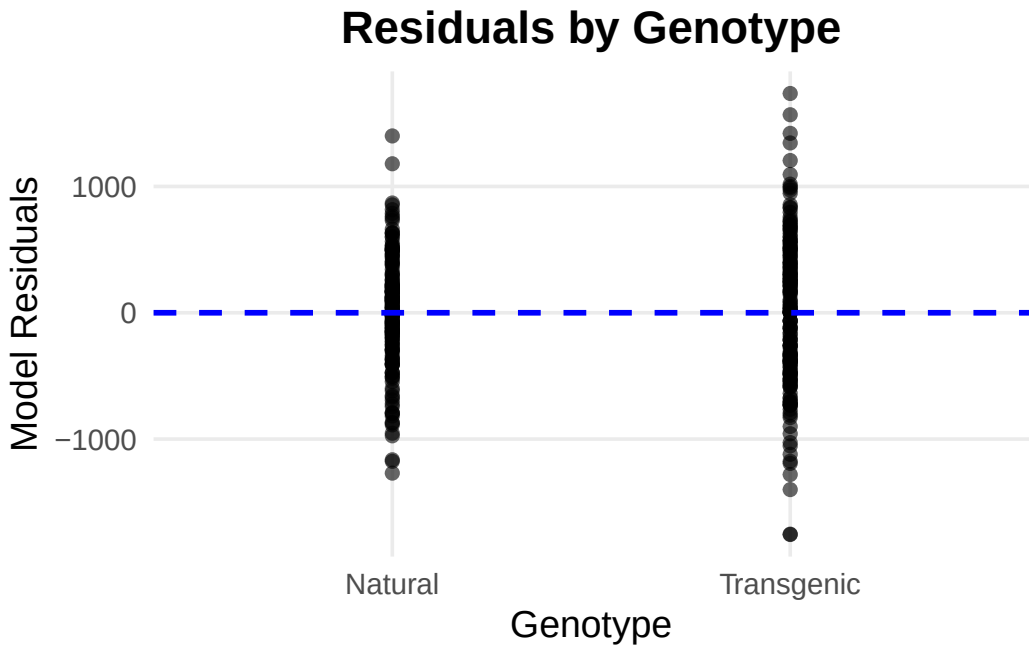
```
SubstratePMOc                          -8026.61    6917.62     310.97  -1.160
SubstratePMPc                         -54350.93    6917.62     310.97  -7.857
naturalTransgenic:Dose                  1513.62     489.53     310.97   3.092
naturalTransgenic:SubstrateOcM        -17567.40   10260.49     310.97  -1.712
naturalTransgenic:SubstratePcM        -10213.84   10260.49     310.97  -0.995
naturalTransgenic:SubstratePM         -11623.31    9782.99     310.97  -1.188
naturalTransgenic:SubstratePMOc         7909.05    9782.99     310.97   0.808
naturalTransgenic:SubstratePMPc        26976.20    9782.99     310.97   2.757
Dose:SubstrateOcM                      -7485.71     513.42     310.97 -14.580
Dose:SubstratePcM                      -7737.11     513.42     310.97 -15.070
Dose:SubstratePM                          26.09     489.53     310.97   0.053
Dose:SubstratePMOc                      -532.46     489.53     310.97  -1.088
Dose:SubstratePMPc                     -3706.61     489.53     310.97  -7.572
naturalTransgenic:Dose:SubstrateOcM    -1278.14     726.09     310.97  -1.760
naturalTransgenic:Dose:SubstratePcM     -784.42     726.09     310.97  -1.080
naturalTransgenic:Dose:SubstratePM      -790.30     692.30     310.97  -1.142
naturalTransgenic:Dose:SubstratePMOc     550.68     692.30     310.97   0.795
naturalTransgenic:Dose:SubstratePMPc    1854.02     692.30     310.97   2.678
                                      Pr(>|t|)
(Intercept)                            < 2e-16 ***
naturalTransgenic                      0.00140 **
Dose                                   < 2e-16 ***
SubstrateOcM                           < 2e-16 ***
SubstratePcM                           < 2e-16 ***
SubstratePM                            0.84626
SubstratePMOc                          0.24681
SubstratePMPc                         6.48e-14 ***
naturalTransgenic:Dose                 0.00217 **
naturalTransgenic:SubstrateOcM         0.08787 .
naturalTransgenic:SubstratePcM         0.32029
naturalTransgenic:SubstratePM          0.23570
naturalTransgenic:SubstratePMOc        0.41945
naturalTransgenic:SubstratePMPc        0.00617 **
Dose:SubstrateOcM                      < 2e-16 ***
Dose:SubstratePcM                      < 2e-16 ***
Dose:SubstratePM                       0.95753
Dose:SubstratePMOc                     0.27757
Dose:SubstratePMPc                    4.23e-13 ***
naturalTransgenic:Dose:SubstrateOcM    0.07934 .
naturalTransgenic:Dose:SubstratePcM    0.28083
naturalTransgenic:Dose:SubstratePM     0.25451
naturalTransgenic:Dose:SubstratePMOc   0.42697
naturalTransgenic:Dose:SubstratePMPc   0.00780 **
```

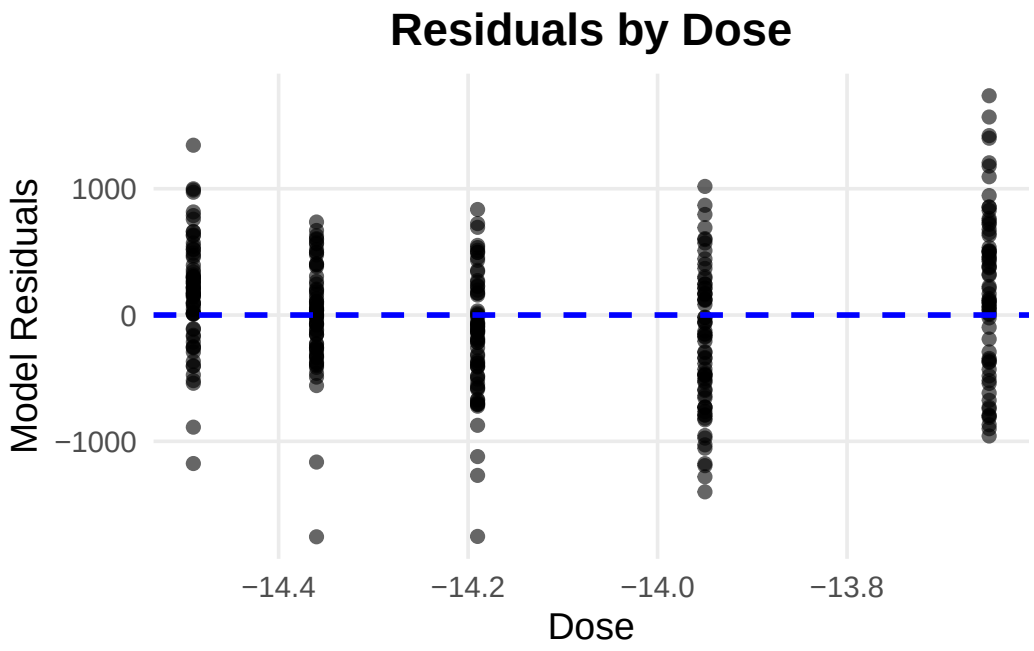
```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


Correlation matrix not shown by default, as p = 24 > 12.
Use print(x, correlation=TRUE)  or
    vcov(x)        if you need it

Type III Analysis of Variance Table with Satterthwaite's method
                          Sum Sq    Mean Sq NumDF  DenDF   F value    Pr(>F)
natural                 17606539   17606539     1 310.97   54.5589 1.397e-12
Dose                  1517357360 1517357360     1 310.97 4701.9667 < 2.2e-16
Substrate              380517369   76103474     5 310.97  235.8284 < 2.2e-16
natural:Dose            15683854   15683854     1 310.97   48.6009 1.886e-11
natural:Substrate        8560933    1712187     5 310.97    5.3057 0.0001077
Dose:Substrate         362539320   72507864     5 310.97  224.6864 < 2.2e-16
natural:Dose:Substrate   8399558    1679912     5 310.97    5.2057 0.0001323

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

refitting model(s) with ML (instead of REML)

Data: data
Models:
lmm2: VO2 ~ Dose * Substrate + (1 | pair)
lmm1: VO2 ~ natural * Dose * Substrate + (1 | pair)
     npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
lmm2   14 5632.8 5686.4 -2802.4   5604.8
lmm1   26 5332.5 5432.0 -2640.2   5280.5 324.39 12  < 2.2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

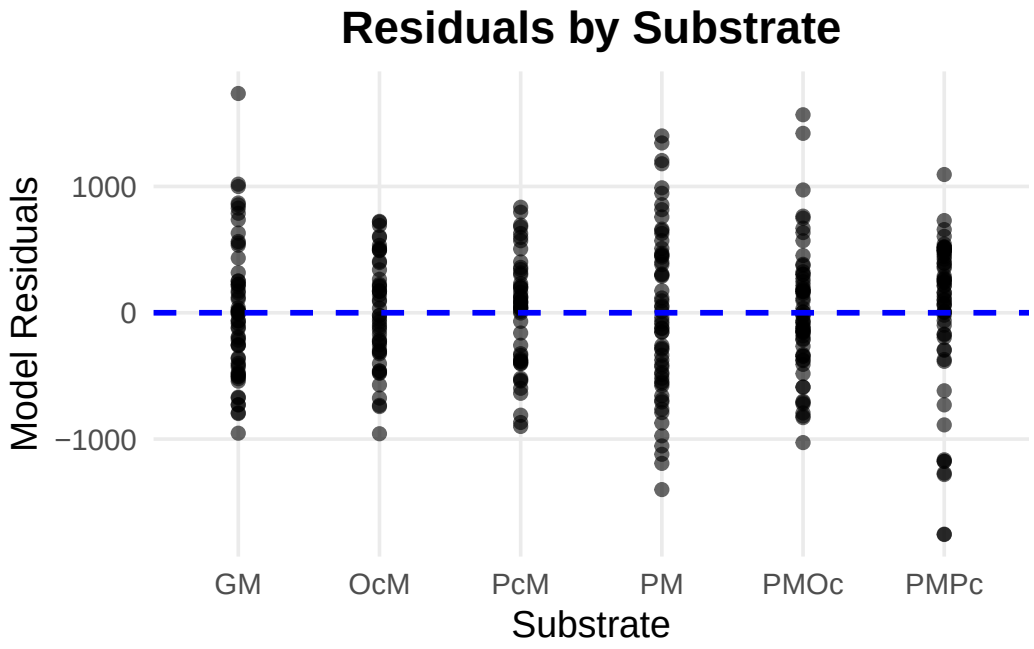
```
Likelihood ratio test

Model 1: VO2 ~ natural * Dose * Substrate + (1 | pair)
Model 2: VO2 ~ Dose * Substrate + (1 | pair)
  #Df  LogLik  Df  Chisq Pr(>Chisq)
1  26 -2489.7
2  14 -2724.9 -12 470.45  < 2.2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

boundary (singular) fit: see help('isSingular')

# R2 for Mixed Models

  Conditional R2: 0.965
     Marginal R2: 0.901

boundary (singular) fit: see help('isSingular')

# Intraclass Correlation Coefficient

    Adjusted ICC: 0.644
  Unadjusted ICC: 0.064



Call:
lm(formula = VO2 ~ natural * Dose * Substrate, data = data)

Residuals:
    Min      1Q  Median      3Q     Max
-3254.1  -370.8   186.2   536.4  1727.3

Coefficients:
                                    Estimate Std. Error t value Pr(>|t|)
(Intercept)                       143278.42    7230.68  19.815  < 2e-16 ***
naturalTransgenic                  22297.32   10225.72   2.181   0.0300 *
Dose                                9596.47     511.68  18.755  < 2e-16 ***
SubstrateOcM                     -109203.79   10724.83 -10.182  < 2e-16 ***
SubstratePcM                     -112973.48   10724.83 -10.534  < 2e-16 ***
SubstratePM                        -1342.39   10225.72  -0.131   0.8956
SubstratePMOc                      -8026.61   10225.72  -0.785   0.4331
```

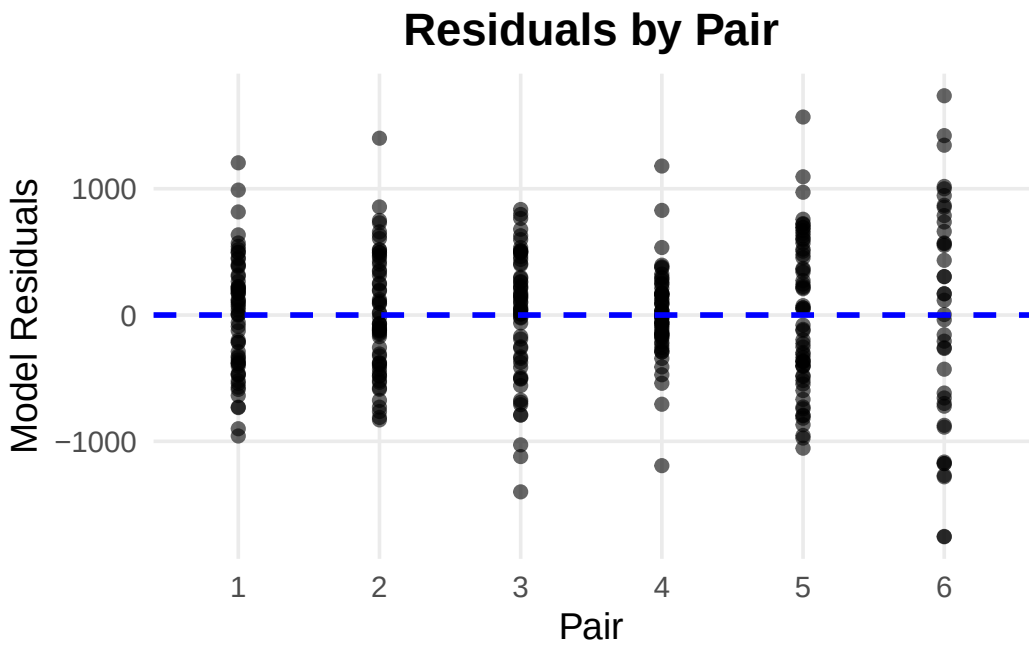
```
SubstratePMPc                          -54350.93   10225.72  -5.315 2.02e-07 ***
naturalTransgenic:Dose                   1513.62     723.63   2.092   0.0373 *
naturalTransgenic:SubstrateOcM         -17567.40   15167.20  -1.158   0.2476
naturalTransgenic:SubstratePcM         -10213.84   15167.20  -0.673   0.5012
naturalTransgenic:SubstratePM          -11623.31   14461.35  -0.804   0.4221
naturalTransgenic:SubstratePMOc          7909.05   14461.35   0.547   0.5848
naturalTransgenic:SubstratePMPc         26976.20   14461.35   1.865   0.0631 .
Dose:SubstrateOcM                       -7485.71     758.95  -9.863  < 2e-16 ***
Dose:SubstratePcM                       -7737.11     758.95 -10.195  < 2e-16 ***
Dose:SubstratePM                           26.09     723.63   0.036   0.9713
Dose:SubstratePMOc                       -532.46     723.63  -0.736   0.4624
Dose:SubstratePMPc                      -3706.61     723.63  -5.122 5.26e-07 ***
naturalTransgenic:Dose:SubstrateOcM     -1278.14    1073.31  -1.191   0.2346
naturalTransgenic:Dose:SubstratePcM      -784.42    1073.31  -0.731   0.4654
naturalTransgenic:Dose:SubstratePM       -790.30    1023.37  -0.772   0.4405
naturalTransgenic:Dose:SubstratePMOc      550.68    1023.37   0.538   0.5909
naturalTransgenic:Dose:SubstratePMPc     1854.02    1023.37   1.812   0.0710 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 839.7 on 316 degrees of freedom
  (20 observations deleted due to missingness)
Multiple R-squared:  0.9233,    Adjusted R-squared:  0.9177
F-statistic: 165.4 on 23 and 316 DF,  p-value: < 2.2e-16


Warning in modelUpdate(objects[[i - 1]], objects[[i]]): original model was of
class "lmerModLmerTest", updated model is of class "lm"


Likelihood ratio test

Model 1: VO2 ~ natural * Dose * Substrate + (1 | pair)
Model 2: VO2 ~ natural * Dose * Substrate
  #Df  LogLik Df Chisq Pr(>Chisq)
1  26 -2489.7
2  25 -2759.2 -1 539.1  < 2.2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

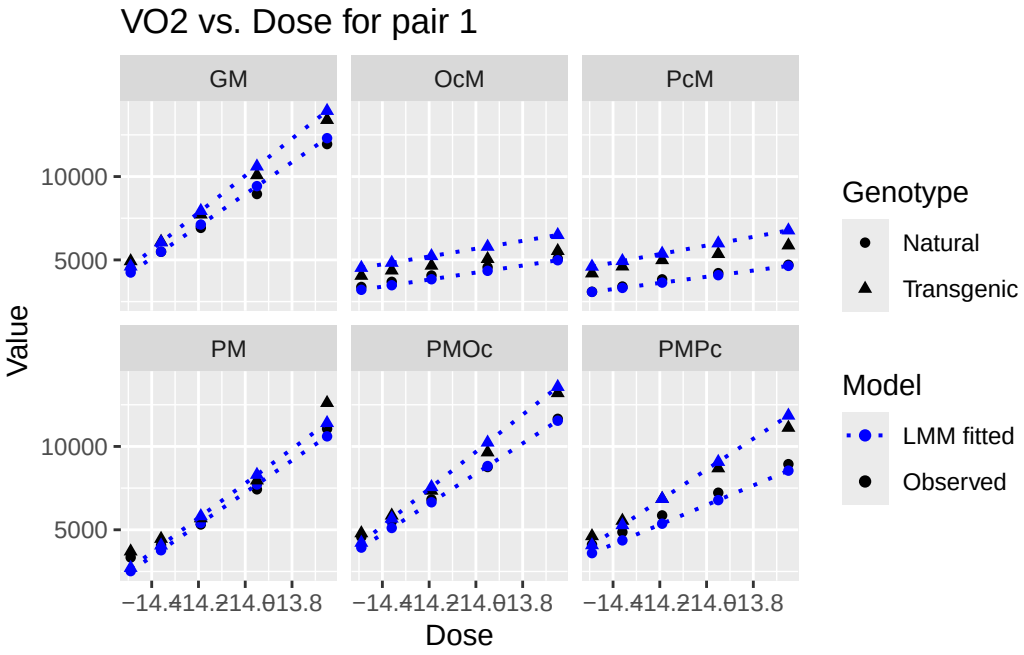

Residuals
Sample Quantiles
1500
1000
500
0
−500
−1000
−1500
−3
−1
1
2
3
Theoretical Quantiles
Random Effects
Sample Quantiles
500
0
−500
−1000
−1500
−1.0
0.0
1.0
Theoretical Quantiles

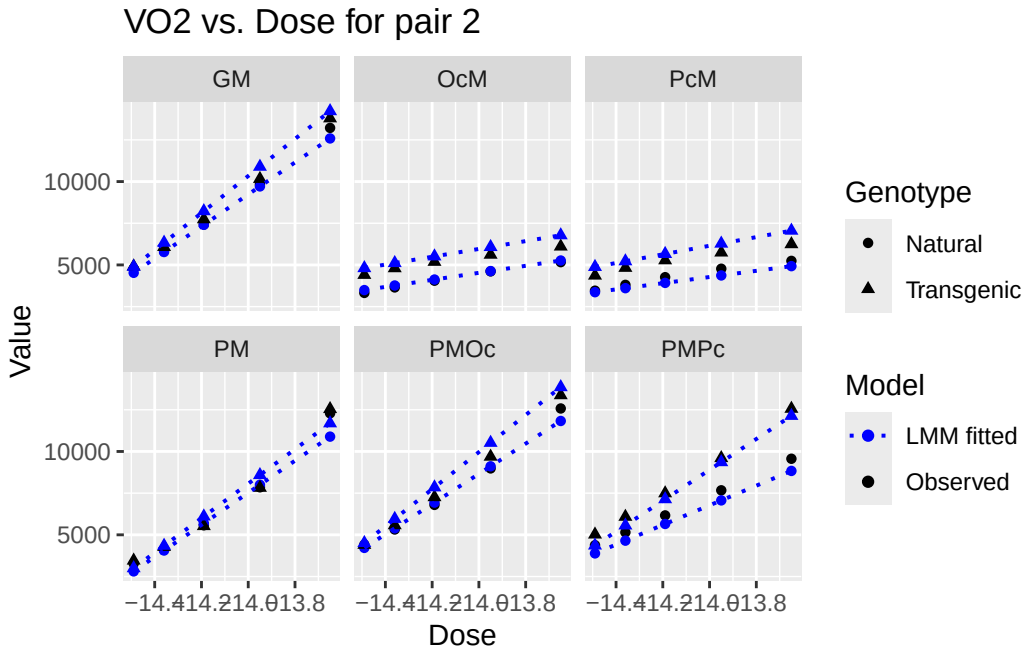

Residuals by Genotype
Model Residuals
1000
0
−1000
Natural
Transgenic
Genotype

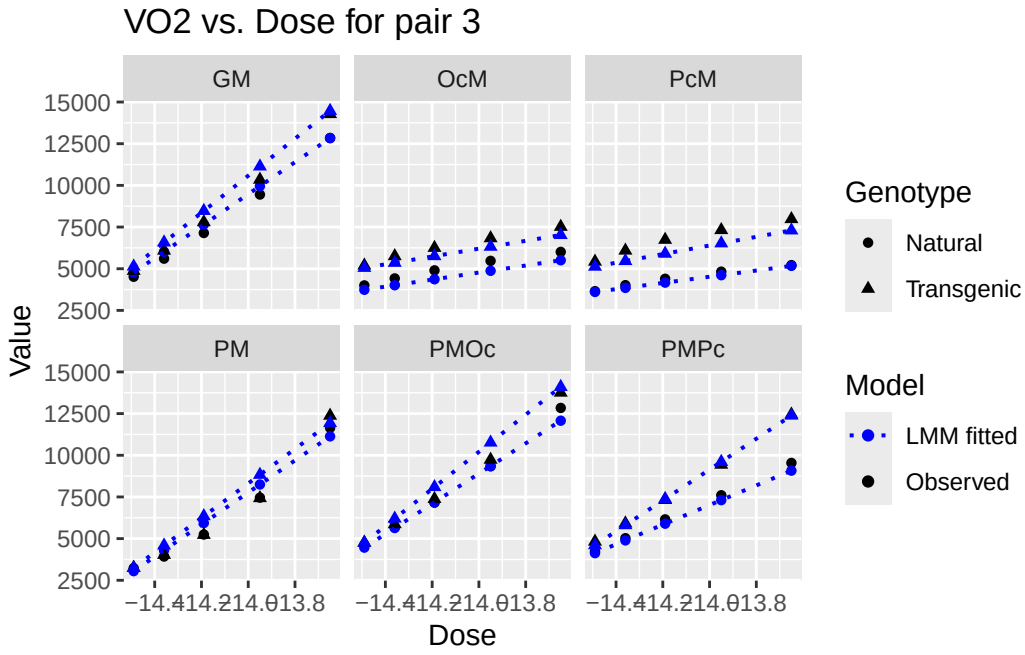

Residuals by Dose
Model Residuals
1000
0
−1000
−14.4
−14.2
−14.0
−13.8
Dose

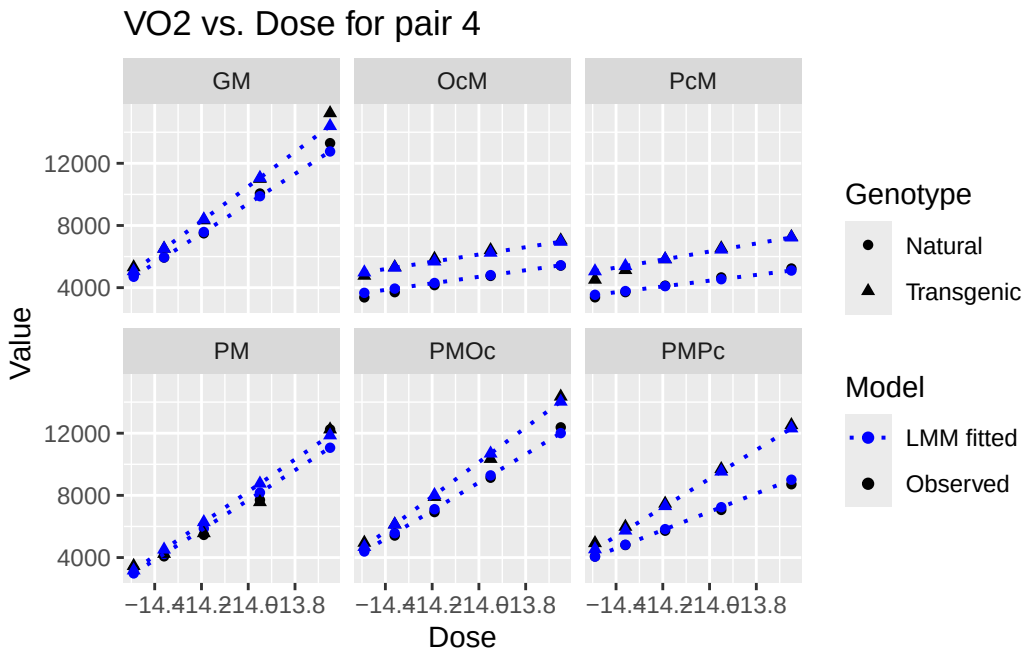

Residuals by Substrate
Model Residuals
1000
0
−1000
GM
OcM
PcM
PM
PMOc
PMPc
Substrate

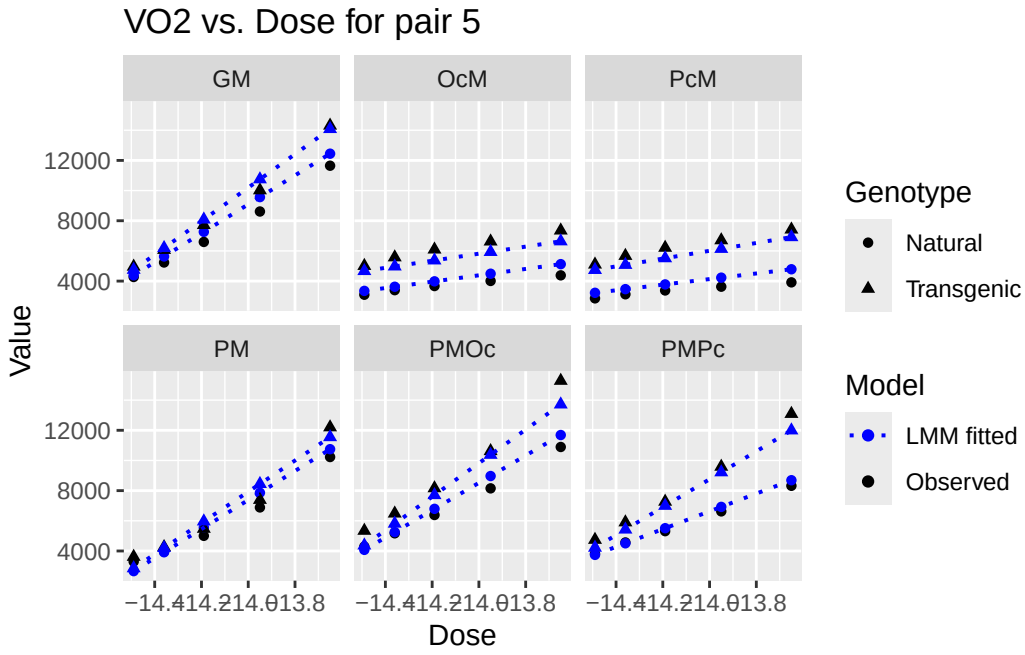

Residuals by Pair
Model Residuals
1000
0
−1000
1
2
3
4
5
6
Pair


VO2 vs. Dose for pair 1
GM
OcM
PcM
PM
PMOc
PMPc
10000
5000
Value
−14.4
14.2
14.0
13.8
Dose
Genotype
Natural
Transgenic
Model
LMM fitted
Observed

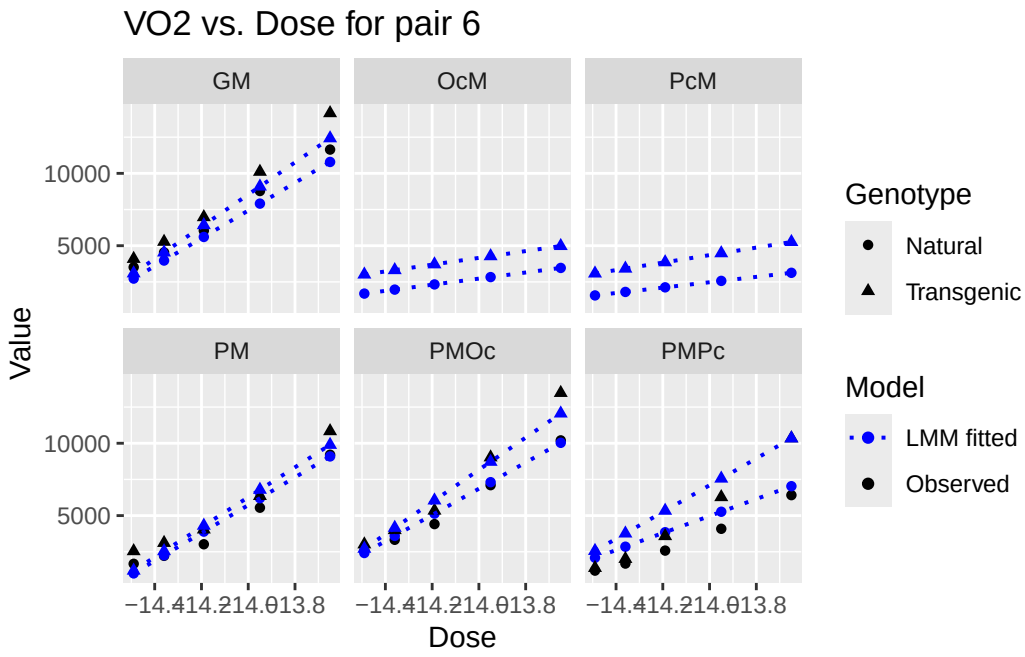

VO2 vs. Dose for pair 2
GM
OcM
PcM
PM
PMOc
PMPc
Value
10000
5000
−14.4
14.2
14.0
13.8
Dose
Genotype
Natural
Transgenic
Model
LMM fitted
Observed

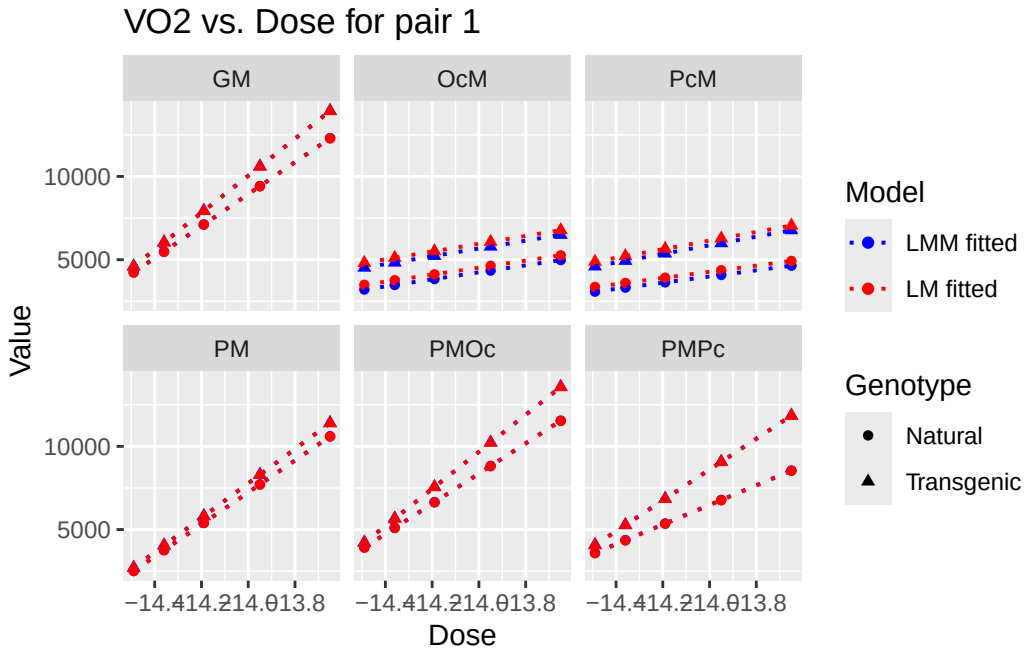

VO2 vs. Dose for pair 3
GM
OcM
PcM
PM
PMOc
PMPc
Value
15000
12500
10000
7500
5000
2500
−14.4
14.2
14.0
13.8
Dose
Genotype
Natural
Transgenic
Model
LMM fitted
Observed

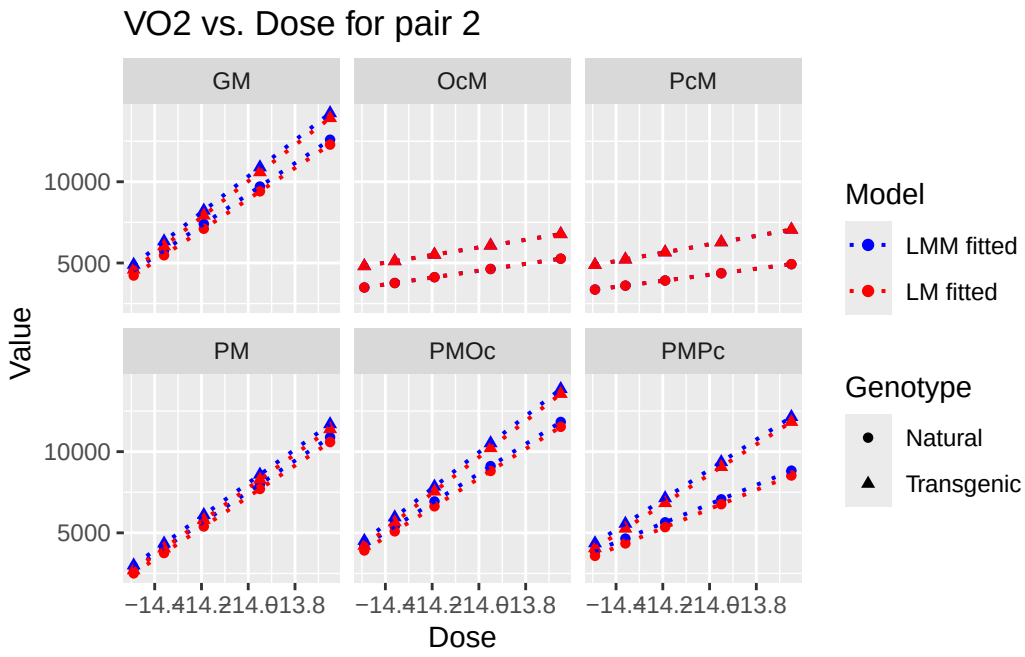


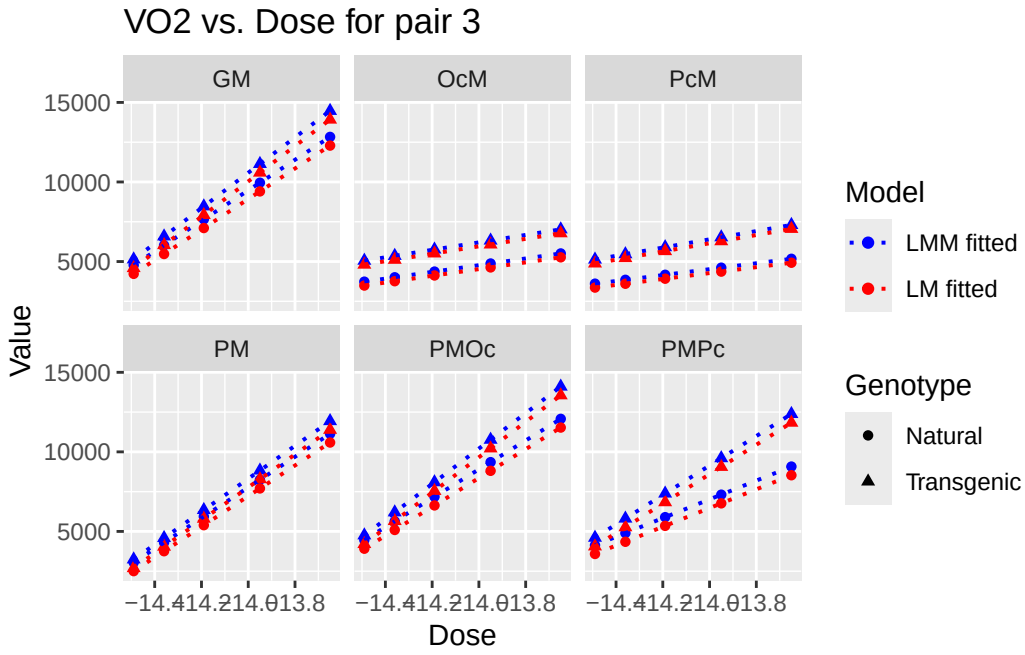


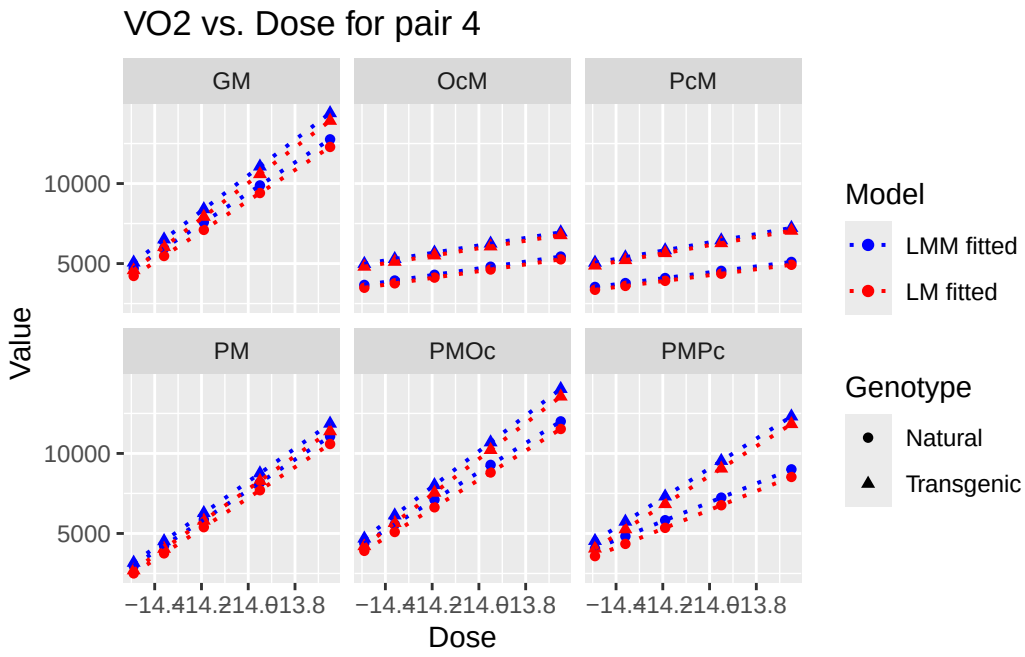

```
Warning: Removed 20 rows containing missing values or values outside the scale range
(`geom_point()`).
```

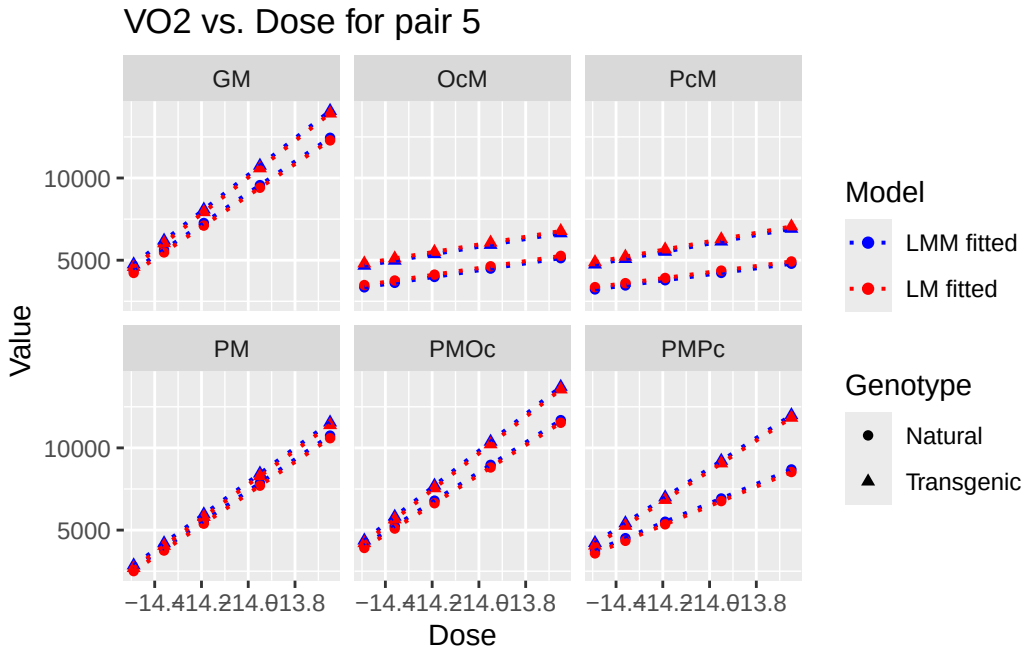

VO2 vs. Dose for pair 6
GM
OcM
PcM
PM
PMOc
PMPc
10000
5000
Value
−14.4
14.2
14.0
13.8
Dose
Genotype
Natural
Transgenic
Model
LMM fitted
Observed

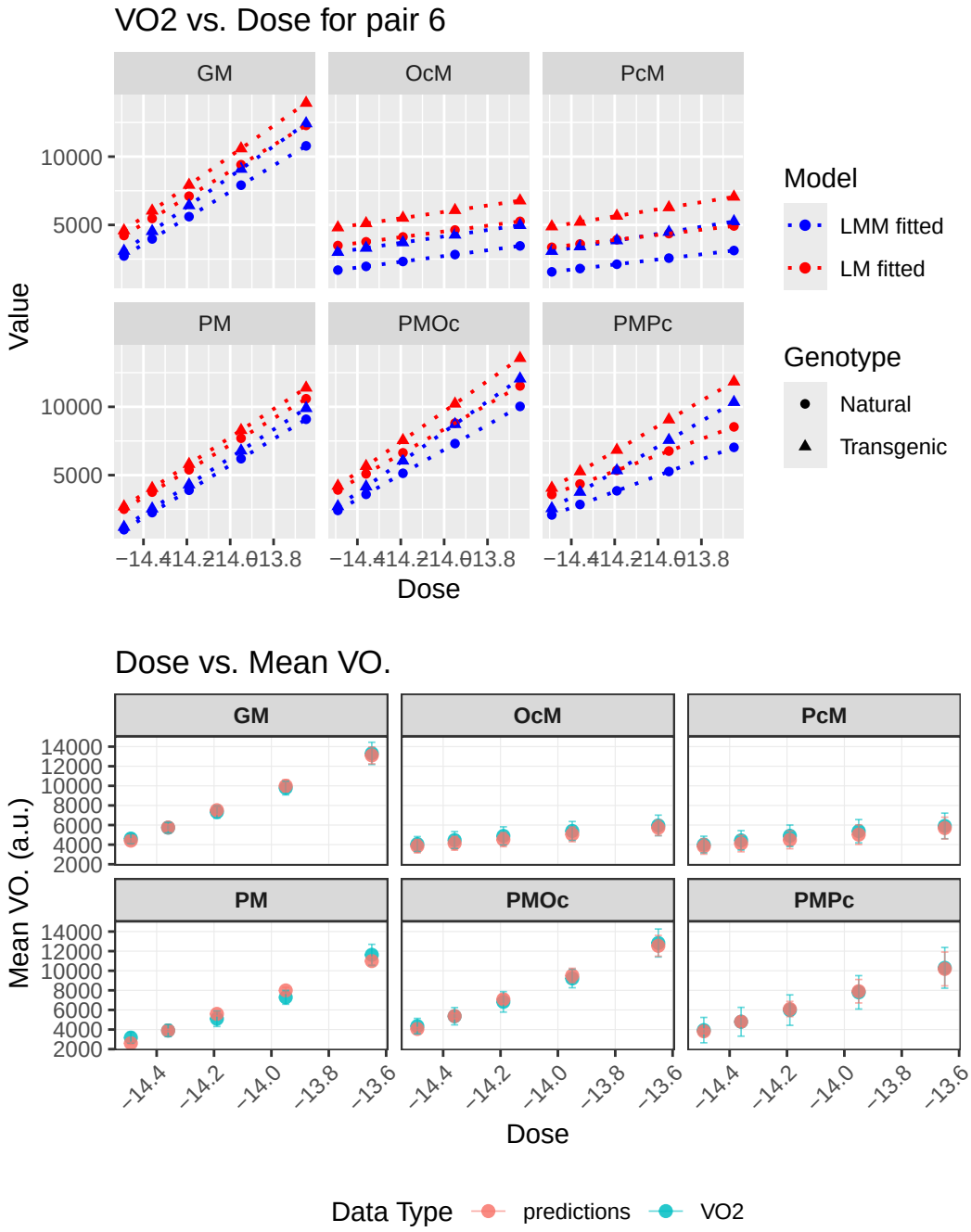

VO2 vs. Dose for pair 1
GM
OcM
PcM
PM
PMOc
PMPc
10000
5000
Value
−14.4
14.2
14.0
13.8
Dose
Model
LMM fitted
LM fitted
Genotype
Natural
Transgenic

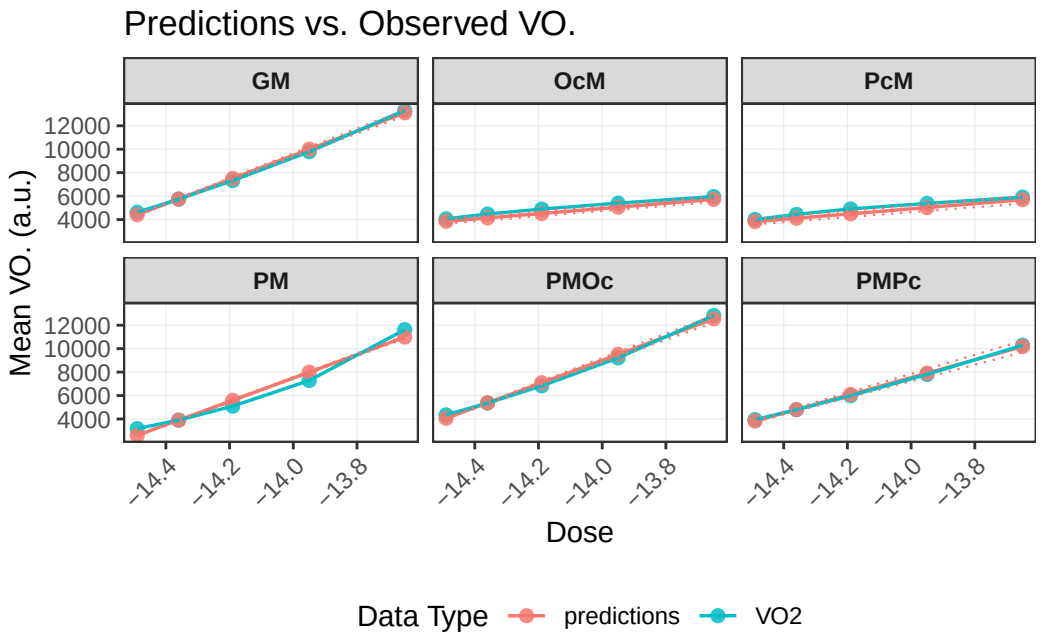

VO2 vs. Dose for pair 2
GM
OcM
PcM
PM
PMOc
PMPc
Value
10000
5000
−14.4
14.2
14.0
13.8
Dose
Model
LMM fitted
LM fitted
Genotype
Natural
Transgenic


VO2 vs. Dose for pair 3
GM
OcM
PcM
PM
PMOc
PMPc
Value
15000
10000
5000
−14.4
14.2
14.0
13.8
Dose
Model
LMM fitted
LM fitted
Genotype
Natural
Transgenic


VO2 vs. Dose for pair 4
GM
OcM
PcM
PM
PMOc
PMPc
10000
5000
Value
−14.4
14.2
14.0
13.8
Dose
Model
LMM fitted
LM fitted
Genotype
Natural
Transgenic


VO2 vs. Dose for pair 5
GM
OcM
PcM
PM
PMOc
PMPc
10000
5000
Value
−14.4
14.2
14.0
13.8
Dose
Model
LMM fitted
LM fitted
Genotype
Natural
Transgenic


VO2 vs. Dose for pair 6
GM
OcM
PcM
PM
PMOc
PMPc
Value
10000
5000
−14.4
−14.2
−14.0
−13.8
Dose
Model
LMM fitted
LM fitted
Genotype
Natural
Transgenic
Dose vs. Mean VO.
Mean VO. (a.u.)
14000
12000
10000
8000
6000
4000
2000
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