Examining the Effect of Antibody Treatment on Muscle Force Measurements in Mice

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November 1, 2022

1 Executive Summary

The client, Dr. Long, conducted an experiment on 40 mice to investigate skeletal muscle biology and the effects of an antibody therapy on muscular function. She wanted to see how much force could be generated with this treatment vs. a control group when volition had no effect on the experiment. In the trials, mice were put to sleep before having a stimulating device implanted in one of their legs. The force of the muscle contraction was then measured after their leg was stimulated and innervated at various frequencies.

This report adequately achieves both of the objectives. First, it is unlikely the administered antibody treatment has a major effect on the force of muscular contraction in mice. Nevertheless, this effect is dependent on gender. Second, there seems to be a negative relationship between contraction rate and relaxation rate. The rates did not vary significantly when grouped by gender. But, when grouped by stimulation frequencies, the rates varied significantly. Smaller stimulating frequencies were associated with higher relaxation rates, whereas larger stimulating frequencies were associated with higher contraction rates.

2 Introduction

2.1 General Background

The most popular mammal used as a model for the human condition is the mouse. Dr. Long conducted an experiment on 40 mice to investigate skeletal muscle biology and the effects of an antibody therapy on muscular function. She wanted to see how much force could be generated with this treatment vs. a control group when volition had no effect on the experiment. Mice were divided into the treatment group and the control group. The assignments were chosen to balance body weight and gender between the two groups. As a result, each group received 20 mice. In the trials, mice were put to sleep before having a stimulating device implanted in one of their legs. The force of the muscle contraction was then measured after their leg was stimulated and innervated at various frequencies.

2.2 Objectives

The primary goal of this consultation is to adequately achieve the following two objectives:

1. **Main Objective**: Identify the effect of the administered antibody treatment on the force of muscular contraction in mice (in terms of force/EDL wt), and determine whether this effect is dependent on gender.

2. **Secondary Objective**: Investigate the relationship between contraction rate and relaxation rate, and determine if these rates are correlated with each other as well as how they vary with gender and stimulating frequency.

2.3 Variable Description

Variable Name	Value(s)
Gender	Male/Female
Treatment	Antibody/Vehicle
Mouse ID	ID number
BW (g)	Mouse's bodyweight
EDL Weight (g)	Innervated muscle weight
EDL Lo (mm)	Optimal length of innervated muscle
Stim Freq (Hz)	1, 10, 20, 40, 60, 80, 100, 150, 250
Max Force (mN)	Total force produced by leg
Force/BW	Force produced per unit weight
Force/EDL Wt	Force produced per unit of muscle weight
Force/EDL Lo	Force produced per optimal length of muscle
Max Rate of Contraction (mN/ms)	Maximum speed of contraction
Max Rate of Relaxation (mN/ms)	Maximum speed of relaxation
Force Frequency	Max force normalized to max force per mouse

3 Project Approach

3.1 Data Cleaning

The client's raw data consists of two distinct data sets: one pertaining to the antibody treatment group and the other to the vehicle group. Processing the raw data involved the following five actions:

1. The variable names in both data sets were made identical.
2. The antibody and vehicle data sets were merged together.
3. Observations with a value of "1e" in the Stim Freq column were omitted since the client is not interested in these observations.
4. In the Stim Freq column, the value "1s" was changed to "1".
5. The Stim Freq column was converted to a factor data type.

3.2 Methodology

We implemented a two-way ANOVA to achieve the **Main Objective**. ANOVA, or Analysis of Variance, is a statistical test used to analyze the difference between the means of more than two groups. A two-way ANOVA is used to estimate how the mean of a quantitative variable changes according to the levels of two categorical variables. We use a two-way ANOVA when we want to know how two independent variables, in combination, affect a dependent variable. ANOVA tests for significance using the F-test for statistical significance. The F-test is a groupwise comparison test, which means it compares the variance in each group mean to the overall variance in the dependent variable. If the variance within groups is smaller than the variance between groups, the F-test will find a higher F-value, and therefore a higher likelihood that the difference observed is real and not due to chance. A two-way ANOVA without interaction tests the following two null hypotheses at the same time:

1. There is no difference in group means at any level of the first independent variable.
2. There is no difference in group means at any level of the second independent variable.

We generated scatterplots to visualize the relationship between contraction rate and relaxation rate to achieve the **Secondary Objective**.

4 Results

As previously stated by the lead consultants, the dominant effect on muscle force measurements is the stimulation frequency. Therefore, to estimate the effects of antibody treatment and gender on force, we should subtract the effect of stimulation frequency. If not, it may hinder our ability to identify these effects. Therefore, for each stimulating frequency, we calculated the median Force/EDL Wt measurement and subtracted it from the Force/EDL Wt column for observations with the corresponding stimulating frequency.

Then, we fitted the following linear regression model and conducted a two-way ANOVA, with the median-adjusted Force/EDL Wt as the response, using the `aov()` function:

$$\hat{y} = \hat{\beta}_0 + \hat{\beta}_1 Gender + \hat{\beta}_2 Treatment$$

Using the `summary()` function, we summarized the ANOVA model. Figure 1 shows Gender is statistically significant while Treatment is not. Thus, we can see Gender explains a significant amount of variation in Force/EDL Wt but Treatment does not.

```
             Df    Sum Sq   Mean Sq F value   Pr(>F)    
Gender        1 8.327e+08 832658683  24.329 1.25e-06 ***
Treatment     1 3.696e+06   3696316   0.108    0.743    
Residuals   357 1.222e+10  34224512                     
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Figure 1: Summary Output of Two-Way ANOVA Model

The scatterplots we generated, grouped by Gender and Stimulating Frequency, are shown in Figures 2 and 3, respectively. The scatterplots show a negative relationship between contraction rate and relaxation rate. When the contraction rate increases, the relaxation rate decreases, and vice versa. We also observe that at high relaxation rates and low contraction rates, the observations are clumped together. However, as the relaxation rate falls and the contraction rate rises, the observations become more dispersed. Figure 2 illustrates the rates did not vary much when grouped by gender. However, Figure 3 illustrates the rates varied when grouped by stimulating frequency. The majority of the clumped up observations had stimulation frequencies of 1, 10, and 20. In contrast, the majority of the dispersed observations had a stimulating frequency of 40, 60, 80, 100, 150, and 250. Furthermore, smaller stimulating frequencies were associated with higher relaxation rates, whereas larger stimulating frequencies were associated with higher contraction rates.

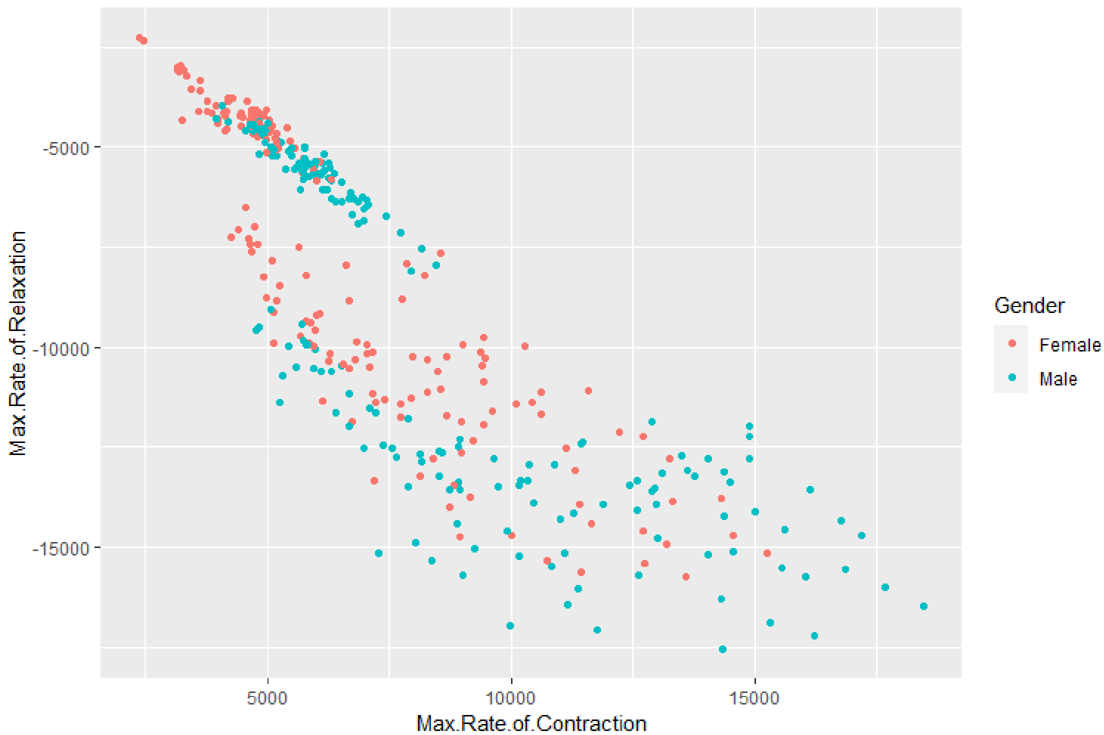

Figure 2: Contraction Rate vs. Relaxation Rate By Gender

Figure 3: Contraction Rate vs. Relaxation Rate By Stimulating Frequency

5 Conclusions

In this report, we feel we have appropriately achieved both objectives. In response to the **Main Objective**, our analysis found Gender to be statistically significant but not Treatment. Therefore, it is unlikely the administered antibody treatment has a major effect on the force of muscular contraction in mice. Nevertheless, this effect is dependent on gender.

In response to the **Secondary Objective**, our analysis found a negative correlation between contraction rate and relaxation rate. Furthermore, at high relaxation rates and low contraction rates, the observations are clumped together. But, as the relaxation rate falls and the contraction rate rises, the observations become more dispersed. When the observations were grouped by gender, the rates did not vary much. However, when the observations were grouped by stimulation frequency, the rates did vary. The majority of the clumped up observations had stimulation frequencies of 1, 10, and 20. Conversely, the majority of the dispersed observations had a stimulating frequency of 40, 60, 80, 100, 150, and 250. Furthermore, smaller stimulating frequencies were associated with higher relaxation rates, whereas larger stimulating frequencies were associated with higher contraction rates.