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Multilevel Models for Longitudinal Data

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Multilevel Models for Longitudinal Data

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A thesis

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In partial fulfillment

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Master of Science in Mathematical Sciences

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by

Aastha Khatiwada

August 2016

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ABSTRACT

Multilevel Models for Longitudinal Data

by

Aastha Khatiwada

Longitudinal data arise when individuals are measured several times during an observation period and thus the data for each individual are not independent. There are several ways of analyzing longitudinal data when different treatments are compared. Multilevel models are used to analyze data that are clustered in some way. In this work, multilevel models are used to analyze longitudinal data from a case study. Results from other more commonly used methods are compared to multilevel models. Also, comparison in output between two software, SAS and R, is done. Finally a method consisting of fitting individual models for each individual and then doing ANOVA type analysis on the estimated parameters of the individual models is proposed and its power for different sample sizes and effect sizes is studied by simulation.

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1 INTRODUCTION

With the intention to further my education in the field of Biostatistics, we decided to work on a thesis topic that is of interest in that area. Biostatistics is a relatively new discipline that largely involves analysis of biological and medical science data sets. Longitudinal data sets are frequently encountered in the field of biostatistics. There are several methods that can be used to analyze longitudinal data sets and it is interesting to compare them. In this work, a relatively new approach of building statistical models, namely, multilevel models, is used to build models and perform analysis for longitudinal data sets.

The second chapter presents an introduction to longitudinal data sets along with some examples to illustrate the composition of such data sets. That chapter also includes different classical approaches that are commonly used in the analysis of longitudinal data sets. Output results for some longitudinal analysis performed on a data set obtained from a case study is also included. Chapter 3 provides a short description about the multilevel models in the regression context along with some supporting examples. A case study to illustrate different types of multilevel model (unconditional and conditional) formation is included in this chapter. In Chapter 4, multilevel models are developed for longitudinal data sets intertwining the results and approaches discussed in the second and third chapter. Output results for a few models along with some suitable graphs are also presented in this section.

In the fifth and final part of the thesis we propose a different method that consists on fitting individual linear models for each individual and then doing a comparison

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of the parameters of those models to see if they vary or not across the groups defined by the treatments. First the method is applied to the mice case study and the estimated slopes of the individual models are compared for the different treatments using ANOVA, Kruskal-Wallis test and the randomization test, for which R code was written. Then the power of this new method is studied doing simulations. Data were simulated using regression models for different scenarios of effect sizes in the slopes and sample sizes. We report the proportion of times that the null hypothesis, of no difference between treatments, was rejected.

2   LONGITUDINAL DATA

Longitudinal data entails taking repeated measurements for the same subject over a period of time [1]. Since data is collected for each subject over time, a longitudinal study involves collecting "repeated measurements" for each subject. Repeated measurement involves recording repeated response outcome for each subject at different periods of time or under different physical conditions [2]. Therefore, longitudinal data analysis branches out from repeated measure analysis.

Analysis of data collected during a longitudinal study is useful in studying over time change for any particular individual. This data can also be used in comparing over time differences between different individuals. Longitudinal data is also useful in analyzing numerous factors that may cause different individuals to respond differently over time. Ability to make these comparisons have been widely appreciated by researchers in social and biological settings. As a result, a significant number of social and biological scientists now use longitudinal data studies as part of their research. In social sciences, longitudinal analysis is most commonly used to learn about developmental trends among living beings or to study social phenomenons like poverty, inequality or drug violence. In biological and life sciences research, longitudinal study is used to learn about microorganisms, different kinds of diseases and so on.

2.1   Examples

An example of longitudinal data would be collecting body weight for children (subjects) prescribed with two different anti-epileptic drugs every week for a year.

This will entail collecting 52 measurements (one for each week of the year) for each child (subject) and using this data set to analyze the changes in weight or the number of seizures as a result of the two prescribed medications. The change in weight comparison can be done for each child or between groups of children receiving different drugs. Also comparisons can be done between children receiving the same drug.

Another example of longitudinal study would be collecting test score results from students who learn English as a second language using one of three available methods. The goal is to monitor English writing skills for non-native speakers. A writing test could be given out to students every month and their scores can be recorded to observe the changes in scores over a period of time for each student. This comparison in scores can also be made between different students to observe how one student's score differs from another student's score and also to compare the three methods separately. This can be helpful in implementing good study techniques to improve English writing skills among students in countries where English is not the first language.

An example of longitudinal data analysis in the context of research in diabetes mellitus (DM) is described in [3]. In this research, there are two factors involved: Streptozotocin (STZ), a DM-inducing drug, and physical restraint. Three levels of STZ (0 mg kg^{-1}, 25 mg kg^{-1} and 50 mg kg^{-1}) and two levels of physical restraints (yes or no) are considered. Therefore, a total of six treatments were considered: 0 mg kg^{-1} STZ with no stress (group 1), 25 mg kg^{-1} STZ with no stress (group 2), 50 mg kg^{-1} STZ with no stress (group 3), 0 mg kg^{-1} STZ with stress (group 4), 25 mg kg^{-1} STZ with stress (group 5), and 50 mg kg^{-1} STZ with stress (group 6). Sixty mice, ten in each treatment group, were used in the research. Measurements

were taken at 0, 2, 4, 7, 9, 11, 14, 16 and 18 days. The response variables are body weight and blood glucose. The design of the study (two factors, fixed effects, factorial design) indicates to use a two-way ANOVA model with interaction for each one of the response variables in any of the days in which measurements were done. However, if we want to analyze all the data for a given response variable, we need to remember that the observations in different days are not independent because they belong to the same mice. This is a typical case of longitudinal data.

2.2 Graphical Representations

Plots should reveal the differences, if any, among groups, but also the changes through time for each individual. The plot given below is a good representation of what longitudinal data looks like. It is called a profile plot. This plot provides information on the mean glucose level for each of the six treatment groups as measured on different days.
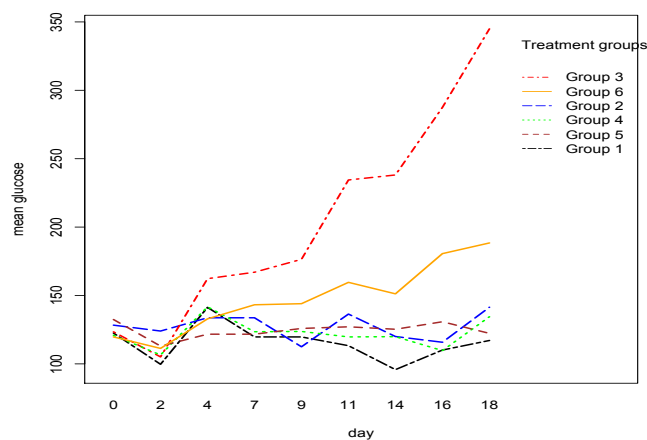
Figure 1: Profile plot for the six treatment groups

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The profile plot in Figure 1 shows that mean glucose levels for treatment groups 1, 2, 4 and 5 are relatively similar on all days . Also, treatment groups 3 and 6 exhibit similar mean glucose levels on days 0, 2 and 4. However, when compared with other treatment groups, mean glucose level for treatment group 3 (50 mg kg^{-1} STZ with no stress) increases at a higher rate day 4 onwards . Also, mean glucose level for treatment group 6 (50 mg kg^{-1} STZ with stress) is relatively higher day 7 onwards when compared to treatment groups 1, 2, 4 and 5.

The plots in Figure 2 are called 'spaghetti' plots. Here, individual plots are drawn for each treatment group. In this plot, data collected over time for each individual subject (mouse) are plotted using individual line segments. This helps us compare the data measurements for all mice on a day by day basis. We can analyze how glucose level for all mice in each treatment levels change over time and also see how glucose level varies for each mice on a daily basis.

Figure 2 indicates that for group 3, glucose level is higher for mice 42, 12, 45 and so on. Similarly, glucose level for mice 30 and 58 in treatment group 6 is much higher on days 14, 16 and 18. The plot also shows that the glucose levels for mice in treatment groups 1, 2, 4 and 5 are approximately on the same levels except for mice 24 and 51 on treatment group 5 . The plots also indicate that even though each group has a certain trend, there is variability among the individuals of each group.
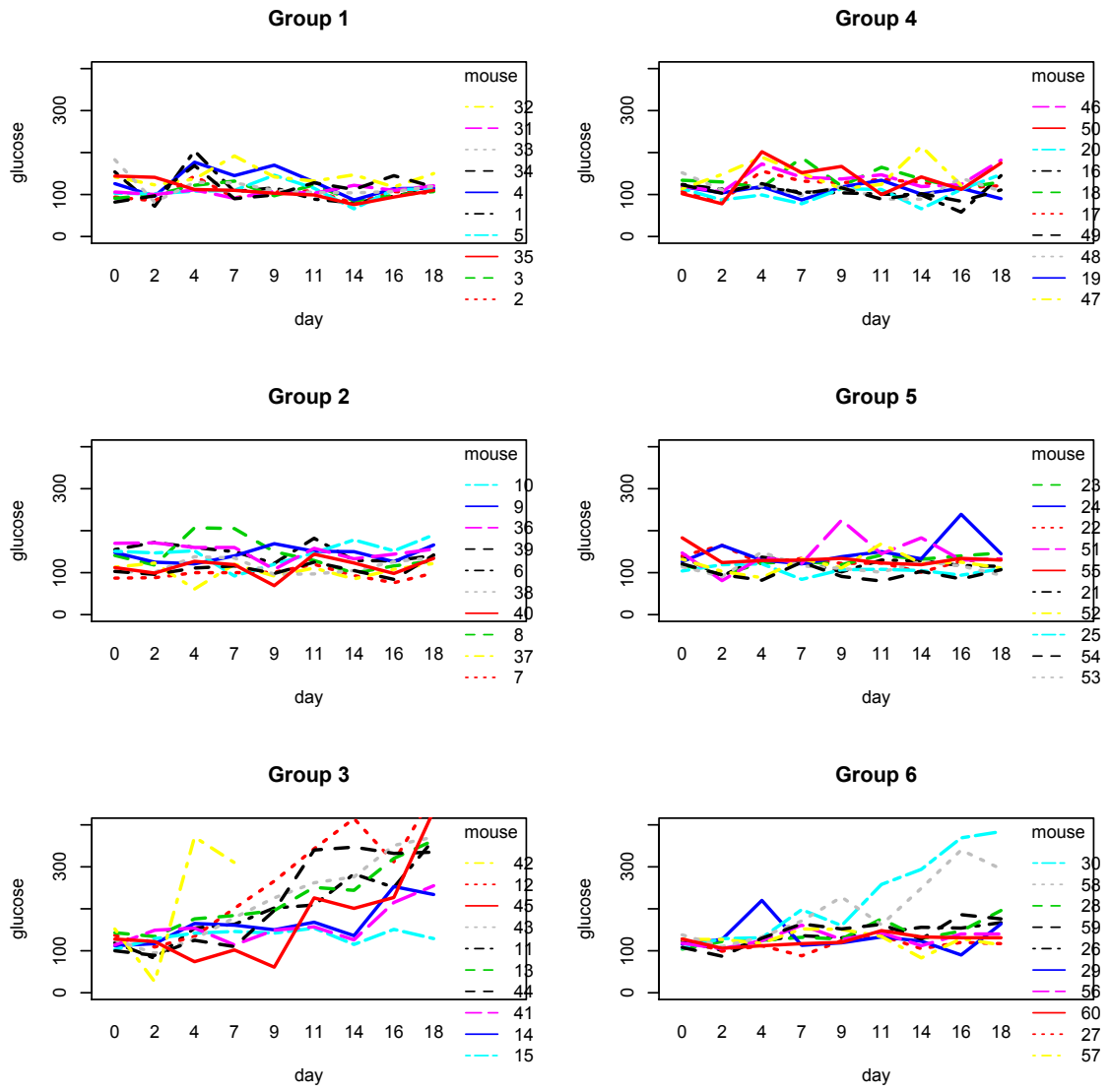
Figure 2: Spaghetti plots for individual subjects in the 6 groups

2.3   Methods to Analyze Longitudinal Data

2.3.1   Mixed Effects Analysis of Variance (ANOVA)

Some of the most common ways of analyzing longitudinal data involves perform-
ing mixed effects analysis of variance (ANOVA) with a single random subject effect
[4]. This is appropriate for the *mice* case study described before. This method is
also widely known as the univariate repeated measures ANOVA. In this method, n
repeated measurements are taken for N individuals. The model is given in Equation
1.

$$Y_{ij} = X'_{ij}\beta + b_i + \varepsilon_{ij}, \qquad i = 1, ..., N; j = 1, ..., n; \tag{1}$$

In Equation 1, Y_{ij} is the outcome of interest, X_{ij} is a design matrix for the fixed
effects, β is a vector of regression parameters, $b_i \sim N(0, \sigma_b^2)$, and $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$.
Here the individual effects (b_i) are random and they represent all the unobserved or
unmeasured factors that make individuals respond differently [6]. There might be
a positive correlation among the repeated measurements for each individual subject
since the observations belong to the same individual.

Mixed effects analysis of variance can be performed on the *mice* data using Equa-
tion 2.

$$Glucose_{ij} = \beta_0 + \beta_1 \times STZ_{ij} + \beta_2 \times Stress_{ij} + \beta_3 \times Day_{ij}$$

$$+ \beta_4 \times STZ_{ij} \times Stress_{ij} + \beta_5 \times STZ_{ij} \times Day_{ij} + \beta_6 \times Stress_{ij} \times Day_{ij}$$

$$+ \beta_7 \times STZ_{ij} \times Stress_{ij} \times Day_{ij} + b_{0i} + \varepsilon_{ij} \tag{2}$$

When performing the analysis, we consider Stress (0 or 1) and STZ (0, 25 and 50) to be fixed effects factors since we wish to see how those specific levels of Stress and STZ influence glucose levels in the mice. The subjects (mice) are random effect factors because they constitute a random sample of individuals where each individual may exhibit a different response to the treatment through time. The R code used to obtain the mixed effects ANOVA table in R using *nlme* package can be written as:

```
am1 = lme(glucose ~ STZ*stress*day, data = mice, random = 1|mouse,
na.action=na.exclude)
anova(am1)
```

The summary of the ANOVA output obtained by using *nlme* package on the DM data for the mixed effects method using Equation 2 is given in Table 1.

This table shows that STZ, Stress, Day and all their interactions are significant.

Table 1: Analysis of variance table for the *micedata* example using R

	numDF	denDF	F-value	p-value
(Intercept)	1	472	1213.1388	<.0001
STZ	1	56	34.5870	<.0001
stress	1	56	5.0538	0.0285
day	1	472	89.4284	<.0001
STZ:stress	1	56	11.1142	0.0015
STZ:day	1	472	151.1998	<.0001
stress:day	1	472	19.7170	<.0001
STZ:stress:day	1	472	44.9351	<.0001

Similarly, the SAS output obtained by using the *PROC MIXED* method on the DM data following Equation 2 is given in Table 2. The code used to generate this output is given as:

```
proc mixed data = mice;

class mouse STZ stress day;

model   glucose  =  STZ  stress  day  STZ*stress  STZ*day  stress*day

stress*STZ*day/S CHISQ;

repeated day/ type=UN subject=mouse R RCORR ;

run;
```

Table 2: Analysis of variance table for the *micedata* example using SAS PROC MIXED

Effect	NumDF	DenDF	Chi-Square	F-Value	Pr>ChiSq	Pr>F
STZ	2	54	44.20	22.10	<.0001	<.0001
stress	1	54	5.63	5.63	0.0176	0.0212
day	8	54	141.60	17.70	<.0001	<.0001
STZ*stress	2	54	14.71	7.35	0.0006	0.0015
STZ*day	16	54	113.36	7.08	<.0001	<.0001
stress*day	8	54	19.53	2.44	0.0123	0.0248
STZ*stress*day	16	54	36.26	2.27	0.0027	0.0131

Similar to the R output, the SAS output also shows that the factors STZ, Stress, Day and all their interactions are statistically significant. The existence of a third order interaction indicates that we should interpret the interaction between STZ and Stress for each day or the behavior of glucose through time for each combination of STZ and Stress.

2.3.2   Multivariate Analysis of Variance (MANOVA)

A second approach to analyzing longitudinal data is using repeated-measures multivariate analysis of variance (MANOVA). In general, MANOVA involves working

with a set of different response variables. In the case of longitudinal data, repeated measurements of the same variable are measured at different times and each one of those measurements play the role of a variable. The mean level of the responses that are collected over time are measured. This data set is then used to answer questions about longitudinal change and its relation to between-subject factors. This method allows flexibility while making assumptions on the structure of covariance among repeated measures and therefore is appealing for statisticians.

The output obtained using MANOVA in R are given in Tables 3 and 4. Table 3 gives the summary for Pillai's test, while Table 4 gives the summary for Wilk's test. These tests help us answer whether there is a significant main effect for Factor STZ or factor Stress and also helps us find out if there is significant interaction between factors STZ and stress. According to Table 3, the Pillai test concludes that mean glucose level differ across different mice based on the amount of STZ given to them. Similarly, stress has no significant impact on glucose levels across different mice. Also, there is no significant interaction between STZ and stress.

Table 3: MANOVA Pillai Test for DM using R

	Df	Pillai	approx F-Val	num Df	den Df	Pr(>F)	
STZ	1	0.49836	5.0777	9	46	$9.056e^{-05}$	***
Stress	1	0.21297	1.3831	9	46	0.2235	
STZ:Stress	1	0.24583	1.6660	9	46	0.1250	
Residuals	54						

Wilk's test also suggests that STZ is the only significant factor and therefore mean glucose level differ across different mice based on the amount of STZ given to them. Stress and the interaction between stress and STZ seem to not have any significant

impact. The result for the Wilk's test is given in Table 4.

Table 4:   MANOVA Wilk's Test for DM using R

```
            Df    Wilks approx    F    num Df   den Df   Pr(>F)
STZ         1       0.50164    5.0777     9       46     9.056e⁻⁰⁵ ***
Stress      1       0.78703    1.3831     9       46     0.2235
STZ:Stress  1       0.75417    1.6660     9       46     0.1250
Residuals   54
```

Likewise, the SAS output for MANOVA for no overall STZ effect is given in Table 5. This table shows that STZ is significant for all different tests (Wilk, Pillai, Hotelling and Roy). Therefore, we can conclude that mean glucose level differ across different mice based on the amount of STZ given to them.

Table 5:   MANOVA different tests to analyze STZ effect on DM data using SAS

```
Statistic                   Value     F Value   Num DF   Den DF   Pr > F
Wilks' Lambda            0.46463245    5.89        9        46     <.0001
Pillai's Trace           0.53536755    5.89        9        46     <.0001
Hotelling-Lawley Trace   1.15223881    5.89        9        46     <.0001
Roy's Greatest Root      1.15223881    5.89        9        46     <.0001
```

Similarly, the SAS output for MANOVA for no overall Stress effect is given in Table 6. This table shows that there is no significant effect of Stress of the mean level of glucose for mice for all four different tests.

Table 6:   Different MANOVA test results to analyze the Stress effect on the *micedata* example using SAS

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.94706014	0.29	9	46	0.9754
Pillai's Trace	0.05293986	0.29	9	46	0.9754
Hotelling-Lawley Trace	0.05589916	0.29	9	46	0.9754
Roy's Greatest Root	0.05589916	0.29	9	46	0.9754

Likewise, the SAS output for MANOVA for interaction between STZ and stress is given in Table 5. This table shows that there is no significant interaction between STZ and Stress for all the four different tests.

Table 7:   Different MANOVA test results to analyze the interaction between Stress and STZ for the *micedata* example using SAS

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.7541	1.67	9	46	0.1250
Pillai's Trace	0.2458	1.67	9	46	0.1250
Hotelling-Lawley	0.3259	1.67	9	46	0.1250
Roy's Greatest Root	0.3259	1.67	9	46	0.1250

It is interesting that the MANOVA approach and the mixed effects ANOVA used in the previous section do not agree on the importance of the effect of Stress and the interaction between Stress and STZ. An alternative method that can be used to analyze longitudinal data involves reducing the sequence of repeated measure data to a single summary value such as the difference between the final and baseline value and then applying the ANOVA method for analysis of univariate response to that single summary value. Since this method compels to focus on only one aspect of the repeated measures over time it limits the scope of the analysis and therefore is not

completely efficient [6]. For this reason, we will not analyze the *mice* data set using this method.

3 MULTILEVEL MODELS

Analyzing data sets that contain variables measured at different levels of hierarchy is known as multilevel modeling. In a multilevel data set subjects in the same level or cluster may be more similar to one another than subjects in other levels or clusters. Multilevel models have extensive use in social sciences. They are more generally used when group level effects need to be analyzed.

3.1 Examples of Multilevel Models

In the regression context, we try to explain one response variable in terms of explanatory variables. If the data set comes from a random sample of a single population, a usual regression model can be applied. For example, we can use a regression model to explain the response variable *'length of hospital stay'* in terms of explanatory variable *'age of the patient'*. We can also use regression to obtain a model to explain how the response variable *'general reading score'* is influenced by the explanatory variable *'vocabulary score'*. However, if the data are clustered because they are selected from different groups defined by other factors, such as patients in 'different hospitals' or students within several 'different schools', the observations might not be totally independent. That is, scores for students in the same school might be correlated because students in the same school have the same teacher or the length of hospital stay for patients in the same hospital might be correlated because hospital policy on patient stay may be same for patients visiting the same hospital and different for patients visiting different hospitals. In such cases, where observations are

separated based on different levels or hierarchy, we use multilevel models instead of using general regression models.

Multilevel models may be used in analyzing standardized test scores for students. In this case, students can be separated based on the schools they attend i.e. students are nested within schools. Therefore, students will represent the first level of the multilevel model and schools will represent the second level of the multilevel model. Students that attend the same school may have similar test scores. In a second scenario, within a school, students may take test preparation classes with different instructors. Then the students that take the test preparation class with the same instructor may have scores that are very similar in nature. In this case, we will have three levels for the multilevel model such that students will be on the first level, instructors will be on the second level and schools will be on the third level.

A second example where multilevel models can be used is in analyzing prevalence of disease among patients admitted in hospitals. In this case, measurements can be collected on patients nested within hospitals. Therefore, patients represent first level of the multilevel model while hospitals represent the second level of the model.

3.2   Multilevel Data Analysis

We will use a multilevel model in the context of regression to analyze a data set related to soil that was provided by Dr. Nandi of the Geoscience department at ETSU [8]. An instance of a multilevel data analysis involves analyzing how acidity of soil (*variable name: pH*) for different locations (*variable name: location*) affect the total exchange capacity of the soil (*variable name: TEC*). There are 6 different locations

- location 1, 2, 3, 4, 5 and 6. The 6 locations are then grouped into 3 different sites based on the kind of vegetation grown on them: sites 1, 2 and 3. The vegetation type grown on locations 1, 2, 5 and 6 are trees ('Alder' in location 1 and 2, and 'Rhododendron' in location 5 and 6) while the vegetation type grown on locations 3 and 4 is grass. Five soil samples were taken from each of the 6 locations and several variables were measured. Here we will work with soil samples that are nested on locations. pH is the independent variable while TEC is the response variable. We will refer to this example as the *soildata* example. Therefore, sample observations are the first level of the multilevel model while location is the second level of the multilevel model. A scatter plot the soil samples nested on locations is given in Figure 3.
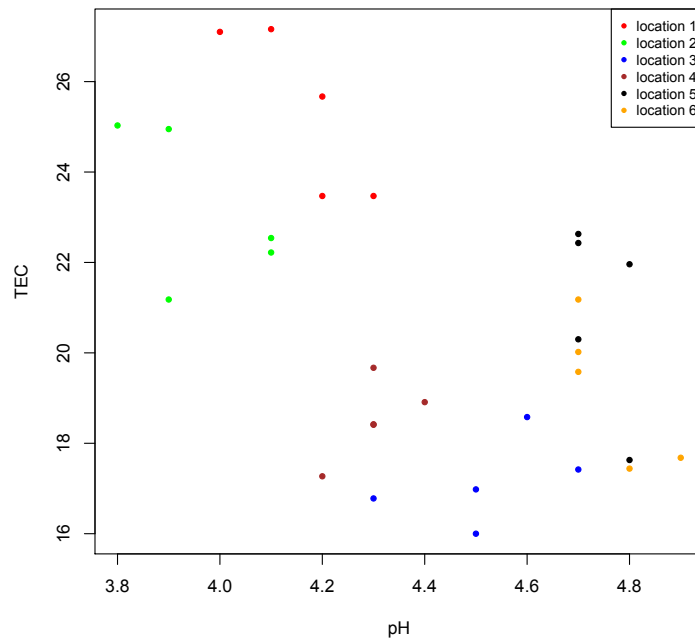
Figure 3: Scatter plot for samples in six locations

We can also compute the correlation between the TEC measure of soil for a given location using the intra-class correlation (ICC). The intra-class correlation is denoted with ρ_I. We can think of ρ_I as the correlation between the TEC measure of soil for two observations randomly selected from the same cluster. For clusters of equal size, ICC can be computed using the formula presented in Equation 3.

$$\hat{\rho}_I = \frac{\hat{\tau}^2}{\hat{\tau}^2 + \hat{\sigma}^2} \tag{3}$$

Here, $\hat{\tau}^2$ represents between cluster sample variance which is the sample variance of TEC measure of soil between the different locations and $\hat{\sigma}^2$ represents within cluster sample variance which is the sample variance of TEC measure within locations. $\hat{\sigma}^2$ is also the variance of ϵ. We can also think of $\hat{\tau}^2$ as the measure of the impact that clusters have on the response variable i.e the impact that locations have on TEC measure of soil. Larger ICC values indicate that observations in the same cluster are more closely related. ICC values are indicative of how clustering may impact the fitted model. Using multilevel data analysis is suggested when large ICC values are obtained [5].

For the *soildata* example, we will use R and SAS to perform multilevel data analysis. For R, two different packages - *nlme* and *lme4* are used.

We will fit two kinds of models here. First, we will fit simple random intercept multilevel models and then we will fit random slope coefficient multilevel models.

3.2.1 Simple Random intercept multilevel models

A random intercept model allows the intercept to vary across different clusters. When we consider a simple linear regression model, one intercept is common between all observations in a data set. However, in multilevel models, data points are grouped together as clusters based on some characteristics such that one common intercept will not suffice for all data points. Therefore, in multilevel models, each cluster will have its own intercept which suggests that the mean for the response variable when the explanatory variable is zero, will vary across the clusters. In this section, we will begin by exploring simple random intercept models known as the null models. Null models are models that do not include any independent variable. Equations for the two levels of a null multilevel model are given in Equations 4 and 5.

$$\text{Level } 1 : y_{ij} = \beta_{0j} + \epsilon_{ij} \tag{4}$$

$$\text{Level } 2 : \beta_{0j} = \gamma_{00} + U_{0j} \tag{5}$$

In Equation 4, the ij subscript is used to denote the ith observation in the jth cluster. So, y_{ij} is the *TEC* value for the ith observation in the jth cluster. β_{0j} is the cluster specific intercept and ϵ_{ij} is the random error term. In Equation 5, γ_{00} denotes intercept effect that is common across all clusters i.e. it is the mean *TEC* value common across all clusters . U_{0j} is the cluster specific effect on the intercept. Since γ_{00} remains constant across all clusters, we can think of it as a fixed effect. Likewise, U_{0j} varies from cluster to cluster, therefore we can think of it as a random

effect.

Equation 6 is obtained by combining Equations 4 and 5 together. Equation 6 provides a framework for simple random intercept multilevel models.

$$y_{ij} = \gamma_{00} + U_{0j} + \epsilon_{ij} \tag{6}$$

We begin our analysis by fitting null models using R and SAS. In R, two different packages *nlme* and *lme4* are used to perform the analysis.

3.2.1.1 Null model using *nlme* package in R

The R syntax for estimating the null model using the *nlme* package is given as:

$$Model1 = \mathbf{lme}(\mathbf{fixed} = response\_variable \sim 1, \mathbf{random} =\sim 1|$$

$$random\_effect\_variable , \mathbf{data} = data\_filename) \tag{7}$$

For our example above, the R code can be written as:

$$Model1 = \mathbf{lme}(\mathbf{fixed} = TEC \sim 1, \mathbf{random} =\sim 1|location , \mathbf{data} = soildata) \tag{8}$$

Summary of the output obtained for *Model1* is given in Table 8.

Table 8: R output for null model using *nlme* package

```
     AIC         BIC          logLik
  132.8804    136.9823    -63.4402
Random effects:
Formula: ~ 1 | location
         (Intercept)    Residual
StdDev:    3.006266     1.576791
Fixed effects: TEC ~ 1
             Value     Std.Error    DF      t-value      p-value
(Intercept)  20.736    1.260614     24      16.44912        0
Standardized Within-Group Residuals:
     Min            Q1          Med            Q3            Max
-2.12251001    -0.76945248    -0.04745117    0.75192944    1.28607833
Number of Observations: 30
Number of Groups: 6
```

The null model provides estimates for between cluster or between location variance $(\hat{\tau}^2)$ and within cluster or within location variance $\hat{\sigma}^2$. From Table 8, we know that $\hat{\tau}^2$ is $3.0063^2 = 9.0376$ and $\hat{\sigma}^2$ is $1.5768^2 = 2.4863$. We can use this output to compute the intraclass correlation (ICC) between the TEC measure of the soil at different locations as in Equation 2. Here, the value would be

$$\hat{\rho}_I = \frac{\hat{\tau}^2}{\hat{\tau}^2 + \hat{\sigma}^2} = \frac{9.0378}{9.0378 + 2.4863} = 0.7843$$

Based on the value above, we can conclude that the correlation of the TEC measure of the soil between places that are in the same location type is 0.7843. Similarly, from the output table, we know that $\hat{\gamma}_{00}$ is 20.736, which is the average *TEC* across all locations.

3.2.1.2 Null model using *lme4* package in R

The R code for estimating the null model using the lme4 package is given below:

$$Model2 = \mathbf{lmer}(response\_variable \sim (1|random\_effect\_variable),$$

$$\mathbf{data} = data\_filename) \quad (9)$$

For our example above, the R code can be written as:

$$Model2 = \mathbf{lmer}(TEC \sim (1|location), \ \mathbf{data} = soildata) \quad (10)$$

Summary of the output obtained for *Model2* is given in Table 9.

Table 9: R output for null model using *lme4* package

```
Linear mixed model fit by REML ['lmerMod']
Formula: TEC ~ (1 | location)
   Data: soildata
REML criterion at convergence: 126.9
Scaled residuals:
     Min        1Q      Median        3Q         Max
  -2.12251   -0.76945   -0.04745    0.75193    1.28608
Random effects:
 Groups Name               Variance      Std.Dev.
 location (Intercept)        9.038         3.006
 Residual                    2.486         1.577
Number of obs: 30, groups:  location, 6
Fixed effects:
             Estimate Std.    Error      t value
(Intercept)      20.736        1.261       16.45
```

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Unlike the *nlme* package, the *lme4* package does not provide the AIC, BIC and log likelihood estimates. Also, the p-value for fixed effects are not included in the output obtained by using the *lme4* package. These values are produced by the *nlme* package. Using *lme4*, p-values can be obtained from the t-value using Markov Chain Monte Carlo (MCMC) method [5]. Apart from that, all other estimates ($\hat{\tau}^2$ is 9.038, $\hat{\sigma}^2$ is 2.486 and $\hat{\gamma}_{00}$ is 20.736) are the same as compared to the output of *Model1* which uses the *nlme* package.

3.2.1.3 Null model using *SAS*

The null model built in SAS uses the same concept as in R - it has no independent variable. The generic SAS code to estimate a null model is given in Table 10.

Table 10: Generic SAS code for null model

```
proc mixed data = data_filename covtest noclprint;
   class random_effect_variable;
   model response_variable = / solution;
   random intercept / subject = random_effect_variable;
run;
```

SAS code for the *soildata* example is given below in Table 11.

Table 11: SAS code for the *soildata* null model

```
proc mixed data = soildata covtest noclprint;
   class location;
   model TEC = / solution;
   random intercept / subject = location;
run;
```

The output obtained from the null model given in Table 11 is given in Table 12.

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Table 12: SAS output for null model

```
            Covariance Parameter Estimates
Cov Parm   Subject   Estimate   StandError  Z Value  Pr > Z
Intercept location    9.0376       6.0321     1.50   0.0670
Residual              2.4863       0.7177     3.46   0.0003
            Fit Statistics
-2 Res Log Likelihood        126.9
AIC (Smaller is Better)      130.9
AICC (Smaller is Better)     131.3
BIC (Smaller is Better)      130.5
            Solution for Fixed Effects
Effect       Estimate   StandError    DF     t Value    Pr > |t|
Intercept    20.7360      1.2606       5      16.45      <.0001
```

Looking at the output, we see that the within cluster $(\hat{\sigma}^2)$ and between cluster $(\hat{\tau}^2)$ variances obtained using the SAS code is the same as that obtained using both *lme* and *lme4* packages in R ($\hat{\tau}^2 = 9.0376$ and $\hat{\sigma}^2 = 2.4863$). Also, the common average affect that all locations have on TEC given by $\hat{\gamma}_{00}$ is 20.7360 which is the same as the R packages. However, the AIC and BIC statistics are lower in the SAS output (130.9 and 130.5 respectively) when compared to the statistics obtained using the *nlme* package (132.89 and 137.0 respectively).

3.2.2 Random slope coefficient multilevel model

Random slope coefficient models are obtained by expanding the simple intercept-only model. This is done by adding independent predictor variables at the individual level (level-1) to simple intercept-only models. Equations for the two levels of the random slope coefficient model can be expressed as:

$$\text{Level 1} : y_{ij} = \beta_{0j} + \beta_{1j}x + \epsilon_{ij} \tag{11}$$

$$\text{Level 2} : \beta_{0j} = \gamma_{00} + U_{0j} \tag{12}$$

$$\beta_{1j} = \gamma_{10} + U_{1j} \tag{13}$$

The level-1 model given by Equation 11 includes an independent predictor variable 'x'. β_{1j} is the slope that relates the independent variable to the response variable i.e it is the change in y that results as a result of a unit change in 'x'. However, β_{1j} is decomposed into two component: γ_{10} which is the average relationship of x with y which is common across clusters and U_{1j} which is the unique cluster-specific variation of the average relationship of x with y. U_{1j} is assumed to have mean 0 and to vary randomly around γ_{10} [5]. The subscript *10* indicates addition of a predictor variable at level-1. ϵ_{ij} is the random error term. β_{0j} in Equation 11 is the mixed intercept effect which is composed of the fixed common intercept effect (γ_{00}) and the random cluster-specific intercept effect (U_{0j}).

Equation 14 is obtained by combining Equations 11, 12 and 13. In this model, $\gamma_{00} + \gamma_{10}x_{ij}$ is the fixed component while $U_{0j} + U_{1j}x_{ij} + \epsilon_{ij}$ is the random component.

$$y_{ij} = \gamma_{00} + \gamma_{10}x_{ij} + U_{0j} + U_{1j}x_{ij} + \epsilon_{ij} \tag{14}$$

While previously the only between cluster variation of U_{0j} was given by $\hat{\tau}^2$ (in Equation 5), now a second source of between cluster variance, given by U_{1j}, is added in Equation 14. Therefore, now we will indicate the variance of U_{0j} by $\hat{\tau_0}^2$ and the

variance of U_{1j} by $\hat{\tau}_1{}^2$. We will also assume that U_{0j} and U_{1j} are independent across different clusters.

When we plot the soil sample clustered by locations and fit a regression line for each cluster, we obtain the plot given by Figure 4. The intercepts and slopes for these regression lines are given in Table 13. Notice that the relationship between TEC and pH is totally different for 4 locations (locations 1, 2, 5 and 6) where trees, namely alder and rhododendron, grow and the 2 locations (locations 3 and 4) where grass grows. The slopes for the locations where tree grows are negative and the slope for the location with grass are positive.
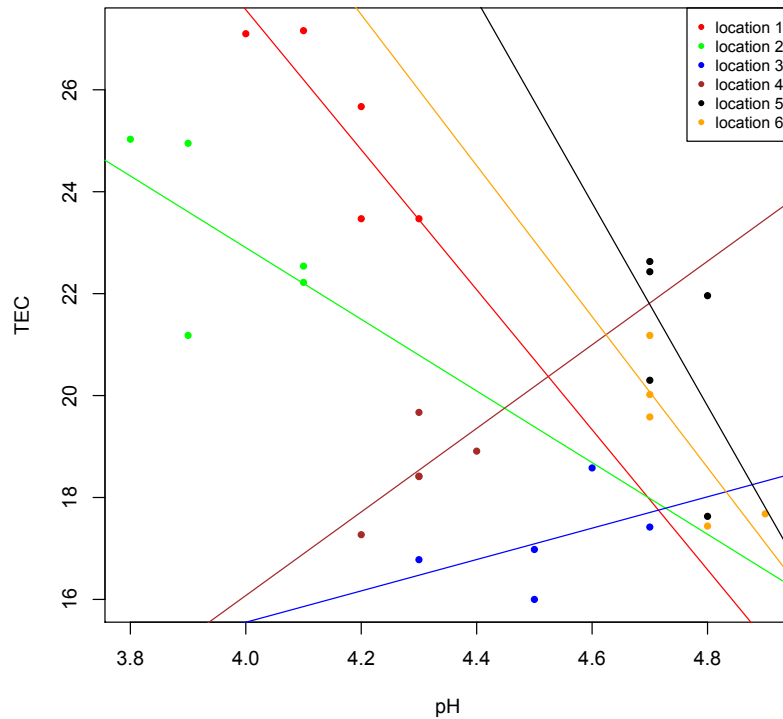
Figure 4: Scatter plot for samples in six locations with regression line

Table 13: Intercepts and slope estimates of multilevel model

Location	Vegetation	Intercept	$\hat{U}_{0j}$	Slope	$\hat{U}_{1j}$
1	Tree (Alder)	82.51	28.32	-13.73	-6.36
2	Tree (Alder)	51.03	-3.16	-7.03	0.34
3	Grass	3.24	-50.95	3.08	10.45
4	Grass	-16.72	-70.91	8.20	15.56
5	Tree (Rhododendron)	115.39	61.20	-19.92	-12.55
6	Tree (Rhododendron)	89.69	35.30	-14.81	-7.44
Overall Mean		54.19		-7.37	

3.2.2.1 Model with one independent variable using *nlme* package in R - random intercept varies within level-1

When we fit a model that includes an independent explanatory variable, the R code can be written as below:

$$Model4 = \textbf{lme}(\textbf{fixed} = response\_variable \sim explanatory\_variable,$$

$$\textbf{random} =\sim 1|random\_effect\_variable, \ \textbf{data} = data\_filename) \quad (15)$$

For the *soildata* example, the R code used to fit the model with pH measure of soil as the independent variable is:

$$Model4 = \mathbf{lme}(\mathbf{fixed} = TEC \sim pH, \ \mathbf{random} = \sim 1|location,$$

$$\mathbf{data} = soildata) \quad (16)$$

In *model4*, **fixed** $= TEC \sim pH$ indicates that the *TEC* value is predicted with the *pH* value being fixed. *random* $= \sim 1|$ *location* indicates that *Model4* is a random intercept model where the random intercept varies by location.

Table 14: R output for full model using *nlme* package

```
     AIC          BIC          logLik
  125.9186    131.2474     -58.9593
Random effects:
 Formula: ~ | location
          (Intercept)        Residual
StdDev:      2.537469        1.484754
Fixed effects: totalexchangecapacity ~ pH
              Value    Std.Error    DF    t-value     p-value
(Intercept)  44.81527  9.890587     23    4.531103    0.0001
pH           -5.46428  2.231267     23   -2.448959    0.0224
Correlation:
   (Intr)
pH -0.994
Standardized Within-Group Residuals:
     Min           Q1            Med           Q3           Max
-1.95260196    -0.59942457    0.07328622    0.71548620   1.14238258
Number of Observations: 30
Number of Groups: 6
```

Table 14 estimates the correlation between the fixed effect slope ($\hat{\gamma}_{10}$) and the fixed effect intercept ($\hat{\gamma}_{00}$) to be -0.994. That is, if we take repeated samples of the two fixed effects - the intercept and the slope for *pH*, the correlation between the two is estimated to be -0.994. The output also shows that after the impact of pH

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measure of soil on TEC is accounted for, the estimated between cluster variance $(\hat{\tau_0}^2)$ for intercept $(\hat{U}_{0j})$ is $2.5375^2 = 6.4387$ and the estimated within cluster variance $(\hat{\sigma}^2)$ is $1.4848^2 = 2.2045$. Similarly, the overall common fixed intercept denoted by $\hat{\gamma}_{00}$, which is the mean TEC value when the pH value is 0, is 44.8153.

Also, observing the output above, we can conclude that the explanatory variable (pH) which is the pH measure of the soil content is a significant predictor of the response variable (TEC) which is the total exchange capacity of soil ($t = $ -2.4490 and p-value $= 0.0224$). Therefore, pH should be included in the model. Interpreting the slope of pH, we can conclude that as pH measure of soil increases by 1 unit, total exchange capacity of soil decreases by 5.4643 units.

We can compare the fit of the model by comparing the AIC and BIC statistics between *Model1* (the null model) and *Model4*. Comparing the AIC and BIC estimates for the null model (*Model1*) and the model with an independent variable (*Model4*), we notice that the values for AIC and BIC are smaller when an independent variable is added to the model. The AIC is reduced from 132.8804 in *Model1* to 125.9186 in *Model4*. Similarly, BIC is reduced from 136.9823 in *Model1* to 131.2474 in *Model4*. This is good as smaller AIC and BIC estimates indicate a better fit. Therefore, this also supports the hypothesis that the independent variable (pH) should be included in the model.

We can also compute R^2 using the output obtained above. In multilevel models, R^2 can be estimated across all levels and it estimates the percentage of variation in the response variable that can be explained by the model at each level. For the

soildata example R^2 can be estimated using the formula:

$$R_1^2 = 1 - \frac{\hat{\sigma}_{M1}^2 + \hat{\tau}_{M1}^2}{\hat{\sigma}_{M0}^2 + \hat{\tau}_{M0}^2}, \tag{17}$$

where $R_1{}^2$ is the R^2 for level-1, the subscript $M0$ indicates null model , therefore $\hat{\sigma}_{M0}^2$ (2.4863) and $\hat{\tau}_{M0}^2$ (9.0376) are the within cluster (within individual) variance and between cluster variance for the null model (model given by *Model1*). Similarly, the subscript $M1$ indicates the model represented by *Model4*. Hence, $\hat{\sigma}_{M1}^2$ (2.2045) and $\hat{\tau}_{M1}^2$ (6.4387) are the within cluster variances and between cluster variances for *Model4*. Plugging in all the values in Equation 16 we get:

$$R_1^2 = 1 - \frac{2.2045 + 6.4387}{2.4863 + 9.0376} = 1 - \frac{8.6432}{11.5239} = 1 - 0.7500 = 0.25$$

Therefore, when compared to the null model or the model without the independent variable (pH), level-1 of *Model4* explains approximately 25 percent more variation in the total exchange capacity of soil (TEC).

3.2.2.2   Model with one independent variable using *lme4* package in R -random intercept varies within level-1

The R code for estimating the model that includes one independent variable using the *lme4* package is given below:

$$model5 = \textbf{lmer}(response\_variable \sim explanatory\_variable +$$

$$(1|random\_effect\_variable), \ \textbf{data} = data\_filename) \quad (18)$$

For our example above, the R code can be written as:

$$model5 = \textbf{lmer}(TEC \sim pH + (1|location), \ \textbf{data} = soildata) \quad (19)$$

The syntax for *lme4* is similar to that for *nlme*. However, a crucial difference lies in how cluster information is included using the random effect variable. In *lme4* there is no specific *random* statement, but the random effect is added next to the explanatory variable in parenthesis. Summary of the output obtained for *Model5* is given in Table 15.

Table 15: R output for full model using *lme4* package

```
Linear mixed model fit by REML ['lmerMod']
Formula: TEC ~ pH + (1 | location)
   Data: mydata
REML criterion at convergence: 117.9
Scaled residuals:
     Min        1Q      Median        3Q        Max
-1.95260   -0.59942    0.07329    0.71549    1.14238
Random effects:
 Groups    Name             Variance    Std.Dev.
 location  (Intercept)        6.439       2.537
 Residual                     2.204       1.485
Number of obs: 30, groups:  location, 6
Fixed effects:
             Estimate    Std. Error     t value
(Intercept)    44.815        9.891         4.531
pH             -5.464        2.231        -2.449
Correlation of Fixed Effects:
   (Intr)
pH -0.994
```

Comparing output for *Model5* with the model output obtained using *nlme* package in *Model4*, we can say that the AIC, BIC and log likelihood and p-value estimates are still missing from the output. However, the results obtained from both packages are the same. We can see that the *scaled residual* five-number summary is identical between the two models. Also, the within cluster ($\hat{\sigma}^2 = 2.204$) and between cluster variance ($\hat{\tau}^2 = 6.439$) are the same. The common fixed intercept effect estimate ($\hat{\gamma}_{00} = 44.815$) and correlation between the two fixed effects (the intercept given by $\hat{\gamma}_{00}$ and the slope given by $\hat{\gamma}_{10}$) is -0.994 which is also the same between the two models.

3.2.2.3 Model with one independent variable using *SAS* -random intercept varies within level-1

SAS code for estimating model with one independent variable (*Model6*) such that random intercept that varies within level-1 is given in Table 16. The only change made in this code compared to the code used to generate the null model in SAS is that an independent variable is added to the model. This is given in the code line:

model response _variable = independent_variable / solution.

Table 16: Generic SAS code for *Model6* that includes an independent variable

```
proc mixed data = data_filename covtest noclprint;
   class random_effect_variable;
   model response_variable = explanatory_variable  / solution ddfm
= bw;
   random intercept / subject = random_effect_variable;
run;
```

The SAS code used for the *soildata* example is given below:

Table 17: SAS code that includes *pH* as an independent variable in the soildata example

```
proc mixed data = soildata covtest noclprint;
   class location;
   model TEC = pH  / solution ddfm = bw;
   random intercept / subject = location;
run;
```

The output obtained for *Model6* using SAS is given in Table 18.

Table 18: SAS output for model including pH as an independent variable

```
            Covariance Parameter Estimates
Cov Parm       Subject     Estimate     StandError    Z Value   Pr > Z
Intercept    location      6.4387        4.4143        1.46     0.0723
Residual                   2.2045        0.6418        3.44     0.0003
          Fit Statistics
-2 Res Log Likelihood           117.9
AIC (Smaller is Better)         121.9
AICC (Smaller is Better)        122.4
BIC (Smaller is Better)         121.5
               Solution for Fixed Effects
Effect       Estimate    StandError       DF      t Value    Pr > |t|
Intercept    44.8153     9.8906           5        4.53      0.0062
pH           -5.4643     2.2313          23       -2.45      0.0224
          Type 3 Tests of Fixed Effects
Effect     Num DF        Den DF      F Value      Pr > F
pH            1            23         6.00        0.0224
```

When the independent variable pH is included in the model, the AIC and BIC estimates obtained using SAS is smaller (121.9 and 121.5 respectively) compared to the output obtained using *nlme* package in R (125.9 and 131.2 respectively). The AIC and BIC estimates are also smaller compared to the outputs obtained for the null models in both SAS and *nlme* package in R.

Also, the within cluster ($\hat{\sigma}^2 = 2.204$) and between cluster variance ($\hat{\tau}^2 = 6.439$) are the same between *Model4, Model5 and Model6*. The common fixed intercept effect estimate ($\hat{\gamma}_{00} = 44.815$) is also the same as the other models. However, the correlation between the two fixed effects (the intercept given by $\hat{\gamma}_{00}$ and the slope given by $\hat{\gamma}_{10}$) is not provided by the SAS output. However, SAS also provides the Type 3 test of fixed effects. This test performs hypothesis testing for the significance of the fixed effect variable pH. Here the F value for the test is 6, which is also equal to square of

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the t-value obtained for pH ($-2.45^2 = 6.0025$). This suggests that the independent variable pH has a significant participation in the model.

3.2.2.4 Model with one independent variable using *nlme* package in R -slope of independent variable varies across level-2 effects

The R code for estimating the model where the impact of the independent variable on the dependent variable (i.e. the slope given by γ_{10}) is allowed to vary across the level-2 effects using the *nlme* package is given below:

$$Model7 = \mathbf{lme}(\mathbf{fixed} = response\_variable \sim explanatory\_variable \;,$$

$$\mathbf{random} =\sim explanatory\_variable \;|random\_effect\_variable),$$

$$\mathbf{data} = data\_filename) \quad (20)$$

In the context of the *soildata* example, for *Model7*, the impact of pH measure of soil on TEC measure of soil varies from one location to another i.e. γ_{10} is included in this model. In this model, we allow both the slope and intercept for the explanatory variable pH to vary randomly from one location to another. The R code in Equation 19 can be modified for the *soildata* as:

$$Model7 = \mathbf{lme}(\mathbf{fixed} = TEC \sim pH\,, \mathbf{random} =\sim pH \mid location),$$

$$\mathbf{data} = soildata) \quad (21)$$

R did not produce an output when this method was used. An error, as given below was generated:

```
Error in lme.formula(fixed = TEC ~ pH, random = ~pH | location,
data = soildata) :
nlminb problem, convergence error code = 1
message = iteration limit reached without convergence (10)
```

This could be because of the small sample size (30). However, an output was obtained when the *lme4* package was used as an alternative. When numerical methods are applied, the efficiency of the different algorithms used by different packages may differ.

3.2.2.5 Model with one independent variable using *lme4* package in R - slope of independent variable varies across level-2 effects

The R code for estimating the model that includes one independent variable using the *lme4* package is given below in *Model8*. In this model, the impact of the independent variable on the dependent variable is allowed to vary across the level-2 effects.

$$Model8 = \textbf{lmer}(response\_variable \sim explanatory\_variable +$$

$$explanatory\_variable \,|random\_effect\_variable), \ \textbf{data} = data\_filename) \quad (22)$$

Equation 21, generated using the *lme4* package is very similar to the equation generation using the *nlme* package. The major difference lies in how *nlme* package assumes the random effects to be correlated or nested in levels while in the *lme4* package the random effects may be either correlated or uncorrelated [5]. the random effects are included inside parentheses in the *lme4* package. For the *soildata* example, the R code using *lme4* package is written as:

$$Model8 \ = \ \textbf{lmer}(TEC \ \sim \ pH \ + \ pH \,|\, location), \ \textbf{data} \ = \ soildata) \quad (23)$$

Summary of the output obtained for *Model8* is given in Table 19.

Table 19: R output for random slope coefficient model using *lme4* package

```
Linear mixed model fit by REML ['lmerMod']
Formula: TEC ~ pH + (pH | location)
   Data: soildata
REML criterion at convergence: 111.5
Scaled residuals:
     Min        1Q       Median        3Q         Max
  -1.88360   -0.41346   -0.05158    0.86013    1.78412
Random effects:
 Groups    Name           Variance    Std.Dev.    Corr
 location (Intercept)    1145.567      33.846
         pH                47.620       6.901     -1.00
     Residual              1.573        1.254
Number of obs: 30, groups:  location, 6
Fixed effects:
            Estimate   Std. Error    t value
(Intercept)   49.629     16.946        2.929
pH            -6.436      3.537       -1.820
Correlation of Fixed Effects:
   (Intr)
pH -0.999
```

Observing the output, we can conclude that the impact of *pH* of the soil on *TEC* of the soil across different locations is not statistically significant (t-value = -1.820). The estimated coefficient for *pH*, is also the same as $\hat{\gamma}_{10}$. This measures the average impact that *pH* has on *TEC* that is common across all locations. Here, $\hat{\gamma}_{10}$ is equal to -6.436. Similarly, $\hat{\gamma}_{00}$ is equal to 49.629. This is the overall common fixed intercept which is the mean *TEC* value when *pH* measure is 0. Also, the correlation between $\hat{\gamma}_{00}$ and $\hat{\gamma}_{10}$ is -0.999.

Similarly, the variance of U_{1j} as given by $\hat{\tau}_1^2$ is estimated to be 47.62. This value reflects the variation in coefficients (slopes) across different locations. Larger $\hat{\tau}_1^2$ estimates indicates that the relationship between the dependent variable (*TEC*) and

the independent variable (pH) is different from one location to another. Similarly, the within cluster variance ($\hat{\sigma}^2$) is equal to 1.573 and the between cluster variance ($\hat{\tau}^2$) is equal to 1145.57. We can see that the largest source of variation in TEC measure of soil comes from the between cluster variance ($\hat{\sigma}^2$) which implies that locations make a difference when estimating a model for the average TEC measure of soil.

3.2.2.6 Model with one independent variable using SAS - slope of independent variable varies across level-2 effects

SAS code for estimating model with one independent variable such that the impact of the independent variable on the dependent variable is allowed to vary across level-2 is given in Table 20. The only change made in this code compared to the code used to generate $Model6$ in SAS is that the independent variable is added to the $random$ part of the model. This is given in the code line: $random\ intercept\ explanatory\ \_variable$ $/\ subject = random\_effect\_variable;$. We will refer to this model as $Model9$.

Table 20: Generic SAS code for $Model9$ that includes an independent variable and a random slope

```
proc mixed data = data_filename covtest noclprint;
   class random_effect_variable;
   model response_variable = explanatory_variable  / solution ddfm
= bw;
   random intercept explanatory_variable/subject=
random_effect_variable type=un;
run;
```

The SAS code used for the $soildata$ example is given in Table 21.

Table 21: SAS code that includes pH as an independent variable in the soildata example

```
proc mixed data = soildata covtest noclprint;
   class location;
   model TEC = pH  / solution ddfm = bw;
   random intercept pH / subject = location type = un;
run;
```

The output obtained for *Model9* is given in Table 22. In this model, pH is used as a level-1 predictor. This variable adds random effect to the model such that its effect can vary across the different locations. We can see that the estimates for the intercept and slope of pH is very close to that obtained using *nlme* and *lme4* package. Also, the covariance estimate of -233.18 with standard error of 276.52 and a *p-value* of 0.3991 suggests that there is no evidence that the effect of pH on the total exchange capacity of soil. The parameter corresponding to UN(2,2) is the variability in slopes of pH. The estimate is 47.1041 with standard error 57.1230. That yields a *p-value* of 0.2048 for one-tailed test. The test is insignificant which suggests that we do not have enough evidence to say that there is some difference in slopes among soil samples at different locations.

This model also has a smaller AIC value (119.4) when compared to *Model6* that does not include pH in the random part of the model. *Model6* has an AIC value of 121.90.

Table 22: SAS output for model with a random slope and pH as an independent variable

```
                  Covariance Parameter Estimates
Cov Parm      Subject      Estimate    StandError   Z Value      Pr Z
UN(1,1)       location      1153.52     1338.89       0.86      0.1945
UN(2,1)       location      -233.18      276.52      -0.84      0.3991
UN(2,2)       location      47.1041      57.1230      0.82      0.2048
Residual                     1.5887       0.4843      3.28      0.0005
                        Fit Statistics
-2 Res Log Likelihood                   111.4
AIC (Smaller is Better)                 119.4
AICC (Smaller is Better)                121.1
BIC (Smaller is Better)                 118.5
                  Solution for Fixed Effects
Effect        Estimate     StandError     DF     t Value     Pr > |t|
Intercept     52.0484       16.9107        5      3.08        0.0275
pH            -6.9761        3.5056       23     -1.99        0.0586
```

Now that we have looked at some multilevel models in a regular setting, in the next section, we will talk about multilevel models in the context of longitudinal data. We will also look at some examples of longitudinal data sets that can be fitted or analyzed using multilevel models.

4 MULTILEVEL MODELS FOR LONGITUDINAL DATA

Multilevel models were developed for analyzing hierarchically structured data. Some examples of hierarchically structured data are students nested within schools and employees nested within companies. Therefore, a hierarchy consists of lower-level observations (individual-level data) nested within higher levels (group-level data).Analysis of models that contain variables measured at different levels of the hierarchy are known as multilevel models [6]. In the case of longitudinal data, multilevel models are useful in the analysis of within person and between person changes by distinguishing two things: how individuals change over time and, how these changes vary across individuals [7].

 In the next part, we will analyze the *micedata* example (from the longitudinal data section) from a multilevel point of view. Here, we will explore whether individual trajectories of change in glucose level differ based on two factors - physical restraint and dose of the drug (STZ) given to them. Empirical change plots with OLS estimated linear trajectories for mice in group 3 (No physical restraint, 50 mg $kg^{-1}$of STZ) is given in Figure 5. Since, this group of mice shows unusually high glucose level (when compared to other groups of mice), we will also analyze this model further in later sections. The plot in Figure 5 shows a linear relationship between between glucose level (y-axis) and the number of days into the experiment (x-axis). This suggests that a linear growth model can be used for level-1 variable 'days'. So, we can write

the linear growth model as given in Equation 24.

$$Y_{ij} = \pi_{0i} + \pi_{1i}Day_{ij} + \varepsilon_{ij} \tag{24}$$

In Equation 24, Y_{ij} is the glucose level of i mouse in group j and Day_{ij} is the day in which measurements are obtained for i mouse in group j. π_{0i} represents individual i's true initial stage i.e. the value of Y_{ij} when Day_{ij} is 0, π_{1i} represents individual i's true rate of change duing the period of study and ε_{ij} represents that portion of individual i's outcome Y_{ij} that is unexplained by Day_{ij} for group j.
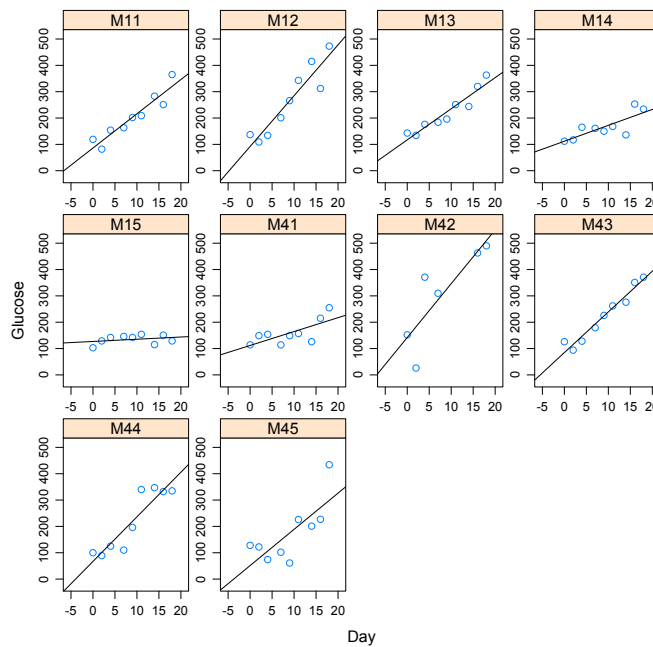
Figure 5: Fitted line for group 3 mice

However, before fitting a model with Day_{ij} as the level-1 predictor, we will first fit a null model i.e. a model without any predictor variable in the next section.

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4.1 The Null Model or Unconditional Means Model (Model A)

The results of this model are helpful in analyzing the partitions in outcome variation i.e. it distinguishes between the individual variation and with-in group variation. The structure of the model is given as:

$$\text{Level 1: } Y_{ij} = \pi_{0i} + \varepsilon_{ij}$$

$$\text{Level 2: } \pi_{0i} = \gamma_{00} + \zeta_{0i}$$

where, $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$ and $\zeta_{0i} \sim N(0, \sigma_0^2)$. Since this model does not contain a slope, the true individual change is a horizontal line with y-intercept π_{0i}. The level-2 equation suggests that, while the flat horizontal lines have different individual departures, their average elevation across everyone in the population, is γ_{00}. So, π_{0i} is the person-specific mean or the true mean of Y for individual i while γ_{00} is the grand mean or the true mean of Y across everyone in the population. So, the level-1 residual ε_{ij} is the deviation in Y_{ij} from individual i's true mean (π_{0i}). Therefore, ε_{ij} is the "within-person" deviation. Similarly, the level-2 residual ζ_{0i} is the deviation in the person-specific mean (π_{0i}) from the average true mean (γ_{00}). Therefore, ζ_{0i} is the "between-person" deviation.

Similarly, variance components σ_ε^2 and σ_0^2 represent the "within-person" variance and "between-person" variance respectively. The "within-person" variance shows how each person's measurements look around their own means while the "between-person" variance shows how a person specific mean looks around the overall population grand mean.

Model A in the next page gives us the result of fitting a null model for the mice

data. The R code executed to fit this model using *lme4* package is given as:

> *ModelL1 = lmer(glucose ∼ 1 + (1 | mouse), data=mice)*
>
> *summary(ModelL1)*

Similarly, the R code executed to fit this model using *nlme* package in R is given as:

> *ModelL2 = lme(glucose ∼ 1, random = ∼ 1 | mouse, data=mice,*
>
> *na.action=na.omit)*
>
> *summary(ModelL2)*

The null model built in SAS uses the same concept as in R - it has no independent variable. The SAS code for the *mice* example is given below in Table 23.

Table 23: SAS code to obtain the null model using *mice* data

```
proc mixed data = mice covtest noclprint;
   class mouse ;
   model glucose = / solution;
   random intercept / subject = mouse ;
run;
```

The results obtained using both packages (*lme4* and *nlme*) in R and in SAS are given in Tables 24, 25 and 26 respectively.

Table 24: Intercept only model using *lme4* package

```
Linear mixed model fit by REML ['lmerMod']
Formula: glucose ~ 1 + (1 | mouse)
   Data: mice
REML criterion at convergence: 5784.9
Scaled residuals:
    Min      1Q    Median      3Q      Max
-5.0050 -0.3953   -0.0775  0.2172   5.4671
Random effects:
 Groups    Name         Variance Std.Dev.
 mouse    (Intercept) 1452       38.11
 Residual             2336       48.33
Number of obs: 536, groups:  mouse, 60
Fixed effects:
            Estimate    Std. Error    t value
(Intercept)  140.592        5.346        26.3
```

Table 25: Intercept only model using *nlme* package

```
Linear mixed-effects model fit by REML
 Data: mice
       AIC        BIC      logLik
   5790.917   5803.764   -2892.458
Random effects:
 Formula: ~1 | mouse
         (Intercept)    Residual
StdDev:      38.1112     48.32862
Fixed effects: glucose ~ 1
             Value    Std.Error    DF    t-value      p-value
(Intercept) 140.592     5.345599   476   26.30051          0
Standardized Within-Group Residuals:
       Min           Q1           Med          Q3          Max
   -5.00499067 -0.39529520 -0.07751821  0.21715801  5.46706819
Number of Observations: 536
Number of Groups: 60
```

Table 26: SAS output for null model

```
              Covariance Parameter Estimates
Cov Parm   Subject   Estimate   StandError  Z Value  Pr > Z
Intercept mouse        1452.46      320.93     4.53   <.0001
Residual               2335.66      151.68    15.40  <.0001
              Fit Statistics
-2 Res Log Likelihood         5784.9
AIC (Smaller is Better)       5788.9
AICC (Smaller is Better)      5788.9
BIC (Smaller is Better)       5793.1
              Solution for Fixed Effects
Effect        Estimate   StandError    DF      t Value    Pr > |t|
Intercept      140.59      5.3456       59       26.30      <.0001
```

Based on the results obtained using both *nlme* and *lme4* package and SAS, we notice that all of them give us the same output values. The estimated grand mean, $\hat{\gamma}_{00}$, is 140.592. This is the overall mean glucose level across all groups of mice. The population mean is different from 0 significantly (t-value of 26.3) which indicates that it should be included in the model. Similarly, the "within-person" variance ($\hat{\sigma}_{\varepsilon}^2$) is 2336 and "between-person" variance ($\hat{\sigma}_0^2$) is 1452. Based on these results we can compute the intra-class correlation coefficient, ρ, which is the proportion of the total outcome variation that lies "between" people. We can write ρ as:

$$\rho = \frac{\hat{\sigma}_0^2}{\hat{\sigma}_0^2 + \hat{\sigma}_{\varepsilon}^2}$$

So, we can calculate ρ by substituting the values of the variance compoenents $\hat{\sigma}_0^2$ and $\hat{\sigma}_{\varepsilon}^2$ as:

$$\rho = \frac{1452}{1452 + 2336} = 0.3833$$

This suggests that about 39 percent of the variation in glucose level can be attributed to differences among individuals. Also, R and SAS produce very similar output results.

In the next few sections we will fit unconditional growth models or models that include explanatory variables. We will begin this analysis by first analyzing only the mice in group 3 as an example.

4.2 Unconditional growth model (Model B) for Group 3 mice

Group 3 includes mice that are not subject to any physical restraint and are given 50 mg kg^{-1} of STZ. For the unconditional growth model we will include days as the level-1 explanatory variable i.e. days change on individual level. So, the structure of the model is given as:

$$\text{Level 1: } Y_{ij} = \pi_{0i} + \pi_{1i} Day_{ij} + \varepsilon_{ij}$$

$$\text{Level 2: } \pi_{0i} = \gamma_{00} + \zeta_{0i}$$

$$\pi_{1i} = \gamma_{10} + \zeta_{1i}$$

where, $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$ and $\begin{bmatrix} \zeta_{0i} \\ \zeta_{1i} \end{bmatrix} \sim N(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \sigma_0^2 & \sigma_{01} \\ \sigma_{10} & \sigma_1^2 \end{bmatrix})$.

Since this model contains a slope, the true individual change has a trajectory. Here, individual i's observed glucose level for group j, Y_{ij}, deviates from the individual's true 'change' trajectory by ε_{ij}. Also, based on the model, we can say that the individual growth parameter (π_{0i} or π_{1i}) is the sum of the intercept (γ_{00} or γ_{10}) and a level-2 residual (ζ_{0i} or ζ_{1i}) [7]. A graphical representation of level-1 model is given in Figure 6.
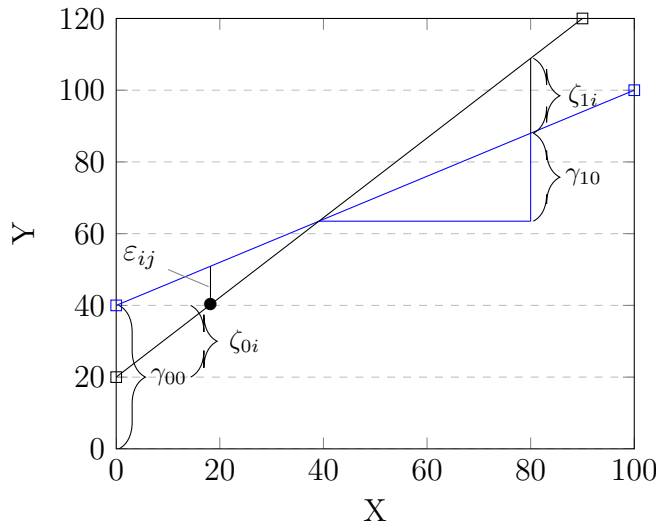
Figure 6: Graphical representation of level-1 model

Also, for this model, interpretation for the variance components σ_ε^2 and σ_0^2 change. The level-1 residual σ_ε^2 which is also the the "within-person" variance now summarizes how each individual's measurements look around his or her own linear change trajectory (this is different than the person-specific mean). Similarly, the level-2 residual variances σ_0^2 (between person variance that comes from intercept) and σ_1^2 (between person variance that comes from slope) now summarize the between person variability in initial status and rates of change. Computing these variances allows us to distinguish between the level-1 variance and the two kinds of level-2 variances.

Model B in the next page gives us the result of fitting an unconditional growth model using 'days' as the explanatory variable for only group 3 mice.

The R code executed to fit this model using *nlme* package is given as:

> *g3glucose = glucose[treatment =="NS50STZ"]*
>
> *g3day = day[treatment =="NS50STZ"]*
>
> *ModelL3 = lme(g3glucose ~ g3day, random = ~ day | mouse,*
>
> *na.action=na.omit)*
>
> *summary(ModelL3)*

Similarly, the R code executed to fit this model using *lme4* package is given as:

> *ModelL4 = lmer(glucose ~ day + (day | mouse), data=mice3)*
>
> *summary(ModelL4)*

Likewise, the SAS code executed to fit this model is given in Table 27.

Table 27: Null model using 'day' as an level-1 independent variable on Group 3 mice in SAS

```
proc mixed data = Group3 covtest noclprint;
   class mouse;
   model glucose =  day/ solution;
   random day / subject = mouse;
run;
```

The results of the fitted models are given in Tables 28, 29 and 30. Table 28 contains the results obtained using the *nlme* package in R while Table 29 contains the output obtained using the *lme4* output in R. Table 30 gives the output produced by SAS.

Table 28: Model using 'Day' as an level-1 independent variable on Group 3 mice using *nlme* package

```
Linear mixed-effects model fit by REML
 Data: NULL
       AIC        BIC        logLik
   951.8178    966.4737     -469.9089
Random effects:
 Formula: ~day | mouse
 Structure: General positive-definite, Log-Cholesky parametrization
             StdDev         Corr
(Intercept) 4.638532e-04     (Intr)
day         5.815610e+00    -0.061
Residual    4.902000e+01
Fixed effects: g3glucose ~ g3day
              Value  Std.Error   DF     t-value       p-value
(Intercept) 98.71005  9.399253    76   10.501904        0
g3day       12.24507  2.036540    76    6.012682        0
 Correlation:
     (Intr)
g3day -0.355
Standardized Within-Group Residuals:
       Min            Q1          Med           Q3           Max
-2.64483919    -0.55094132   0.07178077   0.50308355   3.72281962
Number of Observations: 87
Number of Groups: 10
```

Table 29: Model using 'Day' as an level-1 independent variable on Group 3 mice using *lme4* package

```
Linear mixed model fit by REML ['lmerMod']
Formula: glucose ~ day + (day | mouse)
   Data: mice3
REML criterion at convergence: 939.8
Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.6438 -0.5530  0.0743  0.5031  3.7178
Random effects:
 Groups   Name         Variance  Std.Dev. Corr
 mouse    (Intercept)  3.311e-02  0.1819
          day          3.367e+01  5.8023  1.00
 Residual              2.403e+03 49.0193
Number of obs: 87, groups:  mouse, 10
Fixed effects:
            Estimate Std. Error t value
(Intercept)   98.712      9.399  10.502
day           12.245      2.033   6.024
Correlation of Fixed Effects:
    (Intr)
day -0.350
```

Table 30: Model using 'Day' as an level-1 independent variable on Group 3 mice using SAS

```
            Covariance Parameter Estimates
Cov Parm    Subject    Estimate    StandError  Z Value  Pr > Z
Intercept mouse        33.7899      17.2443      1.96   0.0250
Residual               2403.20      390.33       6.16   <.0001
            Fit Statistics
-2 Res Log Likelihood         939.8
AIC (Smaller is Better)       943.8
AICC (Smaller is Better)      944.0
BIC (Smaller is Better)       944.4
            Solution for Fixed Effects
Effect       Estimate    StandError     DF     t Value   Pr > |t|
Intercept    98.7099      9.3997        76      10.50    <.0001
day          12.2450      2.0358        9        6.01    0.0002
```

Based on the results obtained using both *nlme* and *lme4* package, we notice that both packages give us same output values. Observing the output results, the fixed effect, $\hat{\gamma}_{00}$, is 98.71. This is the overall mean glucose level across all mice in group 3 when slope for Day_{ij} is 0. Similarly, $\hat{\gamma}_{10}$ is 12.25. This is the rate at which Y_{ij} changes for individual i in group 3 when explanatory variable 'Day_{ij}' is included as a level-1 predictor. The fixed effects, $\hat{\gamma}_{00}$ and $\hat{\gamma}_{10}$, estimate the starting point (y-intercept) and slope of the population average change trajectory for group 3 mice. Both parameters are significant (t-value of 10.50 and 6.01 respectively) which indicates that they should both be included in the model. SAS also produces similar output.

Similarly, the "within-person" variance ($\hat{\sigma}_{\varepsilon}^2$) is 2403. This number summarizes the average scatter of an individual's observed outcome values around his or her own true change trajectory [7]. $\hat{\sigma}_{\varepsilon}^2$ is not too different for model B when compared to that for model A (2336). Therefore, the explanatory variable Day_{ij} does not appear to explain a lot of the within-person variance ($\hat{\sigma}_{\varepsilon}^2$) in Y_{ij}. This is also shown by the SAS output in Table 30. Likewise, the level-2 variance components $\hat{\sigma}_0^2$ and $\hat{\sigma}_1^2$ give the amount of unpredictable variation in the individual growth parameters. $\hat{\sigma}_0^2$ is the unpredictable variability in true initial status i.e when day equals 0. It represents the scatter of the $\hat{\pi}_{0i}$ around $\hat{\gamma}_{00}$[7]. The value of $\hat{\sigma}_0^2$ is 3.311×10^{-2}. Similarly, $\hat{\sigma}_1^2$ gives the unpredictable variability in true rates of change. It represents the scatter of $\hat{\pi}_{1i}$ around $\hat{\gamma}_{10}$[7]. Its value is 33.67. Also, this model has an AIC value of 951.8178 which is much lower than the AIC value of 5790.917 that was obtained using the null model in Model A. SAS, on the other hand, produces lower AIC and BIC values.

In the next section, we will look at the unconditional growth model (model B) for

all mice.

4.3   Unconditional growth model (Model B) for all mice

The model used here has the same structure as given in section 4.2 (also given below). However, in this section we will look at all mice instead of focusing in only one group of mice.

$$\text{Level 1: } Y_{ij} = \pi_{0i} + \pi_{1i}Day_{ij} + \varepsilon_{ij}$$

$$\text{Level 2: } \pi_{0i} = \gamma_{00} + \zeta_{0i}$$

$$\pi_{1i} = \gamma_{10} + \zeta_{1i}$$

where, $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$ and $\begin{bmatrix} \zeta_{0i} \\ \zeta_{1i} \end{bmatrix} \sim \text{N}(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \sigma_0^2 & \sigma_{01} \\ \sigma_{10} & \sigma_1^2 \end{bmatrix})$.

The R code executed to fit this model using *nlme* package in R is given as:

```
ModelL5 = lme(glucose ~ day, random = ~ day | mouse, data=mice,
na.action=na.exclude)

summary(ModelL5)
```

Similarly, the R code executed to fit this model using *lme4* package in R is given as:

```
ModelL51 = lmer(glucose ~ day + ( day | mouse), data=mice,
na.action=na.exclude)

summary(ModelL51)
```

Likewise, the SAS code executed to fit this model is given in Table 31.

Table 31: SAS code to fit a model using 'day' as level -1 explanatory variable on complete *mice* data set

```
proc mixed data = mice covtest noclprint;
   class mouse;
   model glucose =  day/ solution;
   random day / subject = mouse;
run;
```

The results of the three fitted models are given in Tables 32, 33 and 34 respectively.

Table 32: Model using 'day' as level -1 explanatory variable on complete mice data set using *nlme* package

```
Linear mixed-effects model fit by REML
 Data: mice
       AIC       BIC     logLik
  5386.427 5412.109 -2687.213
Random effects:
 Formula: ~day | mouse
 Structure: General positive-definite, Log-Cholesky parametrization
            StdDev    Corr
(Intercept) 12.49436 (Intr)
day          5.49093 -0.672
Residual    29.86765
Fixed effects: glucose ~ day
                Value Std.Error  DF  t-value p-value
(Intercept) 117.42164 2.8377908 475 41.37784   0e+00
day           2.63032 0.7413875 475  3.54784   4e-04
 Correlation:
    (Intr)
day -0.566
Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-4.24270447 -0.52093432 -0.03220035  0.34435745  6.02351687
Number of Observations: 536
Number of Groups: 60
```

Table 33: Model using 'day' as level -1 explanatory variable on complete mice data set using *lme4* package

```
Linear mixed model fit by REML ['lmerMod']
Formula: glucose ~ day + (day | mouse)
   Data: mice
REML criterion at convergence: 5384.7
Scaled residuals:
    Min      1Q     Median      3Q       Max
-4.3474  -0.4919   -0.0455   0.3787    5.5059
Random effects:
 Groups    Name         Variance Std.Dev. Corr
 mouse    (Intercept)    0.00     0.000
          day           23.98     4.897      NaN
 Residual               944.49   30.733
Number of obs: 536, groups:  mouse, 60
Fixed effects:
           Estimate Std. Error t value
(Intercept) 117.4255     2.4023    48.88
day           2.6334     0.6705     3.93
Correlation of Fixed Effects:
    (Intr)
day -0.277
convergence code: 0
unable to evaluate scaled gradient
Model failed to converge: degenerate  Hessian with 1 negative
eigenvalues
```

Table 34: Model using 'Day' as an level-1 independent variable on complete mice data set using SAS

```
               Covariance Parameter Estimates
Cov Parm     Subject    Estimate    StandError    Z Value    Pr > Z
day           mouse      23.9624       4.6003        5.21     <.0001
Residual                 944.58       61.3296       15.40     <.0001
               Fit Statistics
-2 Res Log Likelihood          5384.7
AIC (Smaller is Better)        5388.7
AICC (Smaller is Better)       5388.7
BIC (Smaller is Better)        5392.9
              Solution for Fixed Effects
Effect       Estimate    StandError     DF     t Value    Pr > |t|
Intercept     117.43       2.4024       475      48.88    <.0001
day            2.6334      0.6703        59       3.93     0.0002
```

Based on the results obtained using *nlme* and *lme4* package in R, we notice that the fixed effect, $\hat{\gamma}_{00}$, is 117.42. This is the overall mean glucose level across all mice when slope for Day_{ij} is 0. Similarly, $\hat{\gamma}_{10}$ is 2.63. This is the rate at which Y_{ij} changes for individual i when explanatory variable 'Day_{ij}' is included as a level-1 predictor. This is true for mice in all groups. The fixed effects, $\hat{\gamma}_{00}$ and $\hat{\gamma}_{10}$, estimate the starting point (y-intercept) and slope of the population average change trajectory for all mice. Both estimates are significant (t-value of 41.38 and 3.55 respectively) which indicates that they should both be included in the model. This result is also corroborated by the SAS output.

Similarly, R outputs from both packages show that "within-person" variance $\hat{\sigma}_{\varepsilon}^2$ is 892.077 (29.86^2). This number summarizes the average scatter of an individual's observed outcome values around his or her own true change trajectory [7]. $\hat{\sigma}_{\varepsilon}^2$ is still significantly smaller for all mice in Model B than what we obtained for Model A

(2336). Therefore, when analysis is performed on all mice, we can say that a lot of the within-person variance ($\hat{\sigma}_\varepsilon^2$) in Y_{ij} is explained by the explanatory variable Day_{ij}. These output are slightly different for SAS output. The $\hat{\sigma}_\varepsilon^2$ is 944.58 which is slighly higher than that obtained using *nlme* package in R.

Likewise, the level-2 variance components $\hat{\sigma}_0^2$ and $\hat{\sigma}_1^2$ give the amount of unpredicted variation in the individual growth parameters. $\hat{\sigma}_0^2$ is the unpredicted variability in true initial status i.e when day equals 0. It represents the scatter of the π_{0i} around $\hat{\gamma}_{00}$[7]. The value of $\hat{\sigma}_0^2$ is 156.109 (12.49^2). This value is much smaller for just group 3 mice (3.311×10^{-2}). Similarly, $\hat{\sigma}_1^2$ gives the unpredictable variability in true rates of change. It represents the scatter of π_{1i} around $\hat{\gamma}_{10}$[7]. Its value is 30.15 (5.49^2). This value is about the same (33.67) for just group 3 mice.

Also, the AIC and BIC values are comparable between the two software, R and SAS.

In the next section, we will look at unconditional growth models for group 3 and group 6 mice when physical restraint (Stress) is used as a predictor of both initial status and change.

4.4   Unconditional growth models with uncontrolled effects of Stress (Model C) on

Group 3 and Group 6 mice

This model predicts glucose level based on the intercept and Day_{ij} for only group 3 and group 6 mice. It also asks whether the intercept and slope (for Day_{ij}) are affected by physical restraint imposed on mice (Stress $= 0$ or Stress $= 1$). We analyze group 3 and group 6 mice together because group 3 includes mice that do not experience

any physical restraint (Stress $= 0$) and group 6 includes mice that experience physical restraint (Stress $= 1$) while mice in both groups are given the same dose of 50 mg kg^{-1} of STZ. This will help us in understanding how mice that receive the same amount of drug but face different levels of physical restraint behave. The structure of this model looks like:

$$Level\ 1: Y_{ij} = \pi_{0i} + \pi_{1i}Day_{ij} + \varepsilon_{ij}$$

$$Level\ 2: \pi_{0i} = \gamma_{00} + \gamma_{01} \times Stress_i + \zeta_{0i} \qquad (25)$$

$$\pi_{1i} = \gamma_{10} + \gamma_{11} \times Stress_i + \zeta_{1i}$$

where, $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$ and $\begin{bmatrix} \zeta_{0i} \\ \zeta_{1i} \end{bmatrix} \sim N(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \sigma_0^2 & \sigma_{01} \\ \sigma_{10} & \sigma_1^2 \end{bmatrix})$.

The R code executed to fit this model using *nlme* package in R is given as:

```
ModelL6 = lme(glucose ~ day * stress, random = ~ day | mouse, data=gr3gr6,
na.action=na.omit)
summary(ModelL6)
```

The R code executed to fit this model using *lme4* package in R is given as:

```
ModelL7 = lmer(glucose ~ day * stress + (day | mouse), data=gr3gr6)
summary(ModelL7)
```

Likewise, the SAS code executed to fit this model is given in Table 35.

Table 35: SAS code for mice data set using 'day' as the explanatory variable

```
proc mixed data = grp3grp6 covtest noclprint;
   class mouse;
   model glucose =  day stress day*stress/ solution;
   random day / subject = mouse;
run;
```

The results of the fitted models are given in Tables 36, 37 and 38 respectively.

Table 36: Analysis result using 'day' as explanatory variable in *nlme* package for group 3 and group 6 mice

```
Error in lme.formula(glucose ~ day * stress, random = ~day | mouse,
data = gr3gr6, :nlminb problem, convergence error code = 1
message = iteration limit reached without convergence (10)
```

As we can see, the output could not be computed using *nlme* package in R because of convergence problems. The *lme4* package, on the other hand, provides some output results which is given in Table 37.

Table 37: Model using 'day' as explanatory variable in *lme4* package for group 3 and group 6

```
Linear mixed model fit by REML ['lmerMod']
Formula: glucose ~ day * stress + (day | mouse)
   Data: gr3gr6
REML criterion at convergence: 1833.1
Scaled residuals:
    Min      1Q     Median      3Q       Max
-3.4034   -0.4224   -0.0138    0.3708    4.9419
Random effects:
 Groups    Name          Variance    Std.Dev.   Corr
 mouse    (Intercept)    33.35        5.775
          day            31.34        5.598     -1.00
 Residual                1487.71     38.571
Number of obs: 177, groups:  mouse, 20
Fixed effects:
            Estimate    Std. Error    t value
(Intercept)   98.653      7.618        12.950
day           12.244      1.899         6.446
stress        14.479     10.761         1.346
day:stress    -8.383      2.685        -3.122
Correlation of Fixed Effects:
           (Intr)     day      stress
day        -0.514
stress     -0.708    0.364
day:stress  0.364   -0.707     -0.515
```

Also, an AIC value of 1849.085 is generated for *ModelL7* using the codes given below:

```
aic1 = AIC(logLik(ModelL7))

aic1
```

The SAS output result is given in Table 38. There is some difference between the SAS and R output results. But, for the most part, the outputs are very similar to each other.

Table 38: Model using 'Day' as an level-1 independent variable on Group 3 and 6 mice data set using SAS

```
              Covariance Parameter Estimates
Cov Parm     Subject    Estimate    StandError    Z Value    Pr > Z
day           mouse      24.5852      8.9655        2.74      0.0031
Residual                 1541.60      174.61        8.83      <.0001
              Fit Statistics
-2 Res Log Likelihood          1835
AIC (Smaller is Better)        1839
AICC (Smaller is Better)       1839
BIC (Smaller is Better)        1840.8
              Solution for Fixed Effects
Effect       Estimate    StandError     DF      t Value    Pr > |t|
Intercept    98.7323      7.5285        156      13.11      <.0001
day          13.0258      1.7976         17       7.25      <.0001
stress       14.3993     10.6336        156       1.35      0.1777
day*stress   -9.1651      2.4849        156      -3.69      0.0003
```

Substituting the $\hat{\gamma}$ parameter estimates obtained in Tables 36, 37 and 38 in the level-2 sub-model given in Equation 25, we can write:

$$\widehat{\pi_{0i}} = 98.653 + 14.479\widehat{Stress_i}$$

$$\widehat{\pi_{1i}} = 12.244 - 8.383\widehat{Stress_i}$$

(26)

We can then write the composite model as:

$$\widehat{Glucose_{ij}} = 98.653 + 14.479\widehat{Stress_i} + 12.244\widehat{Day_{ij}} - 8.383\widehat{Stress_i}\widehat{Day_{ij}} \qquad (27)$$

The first part of Equation 26 given by $\hat{\pi}_{0i}$ represents the effects of level-2 variable Stress when Day_{ij} is 0 while the second part of Equation 26 given by $\hat{\pi}_{1i}$ represents the effects that level-2 variable Stress has on the slope of level-1 variable Day_{ij}.

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Now, interpreting the $\hat{\gamma}$ parameter estimates given in Equation 26, we can say that the average glucose level for mice in group 3 and group 6 at initial status (Day 0) when no physical restraint is given to them (Stress = 0) is 98.653. This is also the average glucose level for group 3 mice since only group 3 mice receive no physical restraint. However, for mice that receive physical restraint (Stress = 1), the average glucose level is estimated to be 113.132 which is about 14.479 higher than that for mice that do not receive physical restraint. This is also the average glucose level for group 6 mice since only group 6 mice receive physical restraint.

In this part, we will analyze the second part of Equation 26 given by π_{1i}. This part gives the coefficients for the rates of change. Based on the coefficients given, we can say that the average rate of change in glucose level for mice that do not receive any physical restraint (mice in group 3 such that Stress = 0) is 12.244. However, the average rate of change in glucose level for mice that receive physical restraint (mice in group 6 such that Stress = 1) is estimated to be lowered by 8.383 points (at 3.861). Hence, we can say that, on the next day that the glucose level is measured, for mice that do not receive physical restraint, average glucose level goes up by 12.244 points while for mice that receive physical restraint, average glucose level goes up by only 3.861 points. This suggests that imposing physical restraint on mice decreases their blood glucose level. Figure 7, which represents the results of the fitted multilevel model for change, supports the numerical interpretation. Even though the trajectory for mice that do not face any physical restraint (Stress = 0) is incomplete because of missing data, we can interpolate that this trajectory shows an increasing trend and the average glucose level is much higher for mice that do not experience physical restraint.

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This plot also shows that for mice that do not experience any physical restraint, the average glucose level is around 98 while for mice that experience physical restraint, the average glucose level is around 113.
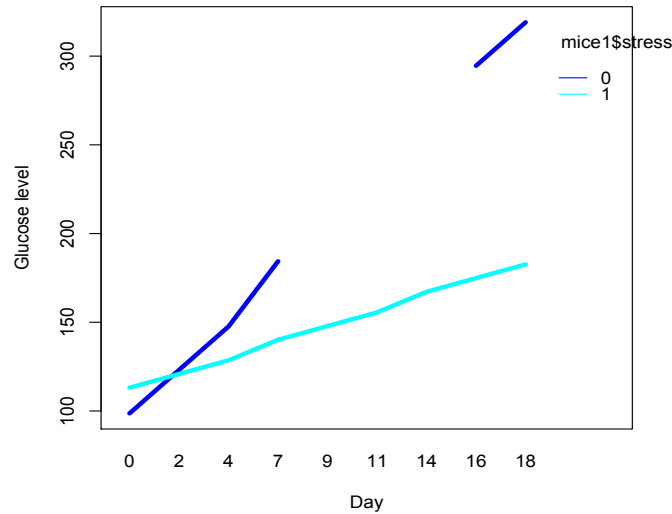
Figure 7: Graphical representation of a fitted multilevel model given in Equation 24 for group 3 and group 6 mice

Now, we will analyze the variance components or the random effects variances obtained in Tables 36, 37 and 38. Variance components give us the amount of outcome variability that are unexplained after fitting the multilevel model given in Equation 25. Based on the results obtained, $\hat{\sigma}_\epsilon^2$, which gives the variability in an average mouse's outcome around it's own true change trajectory, is 1487.71. This is also known as the level-1 residual variance. Similarly, the level-2 variance components as given in Equation 23 as $\begin{bmatrix} \hat{\sigma}_0^2 & \hat{\sigma}_{01} \\ \hat{\sigma}_{10} & \hat{\sigma}_1^2 \end{bmatrix}$ is given by $\begin{bmatrix} 33.35 & -32.33 \\ -32.33 & 31.34 \end{bmatrix}$. So, $\hat{\sigma}_0^2$, which gives the population residual variability of the true initial status (i.e. average intercept when $Day_{ij} = 0$) when controlling for physical restraint (Stress =0 or 1), is about 33.35.

Similarly, $\hat{\sigma}_1^2$, which gives the population residual variance of true rate of change (i.e. slope for Day_{ij}) when controlling for physical restraint (Stress = 0 or 1), is 31.34. And $\hat{\sigma}_{01}$ which gives the population residual covariance between true initial status ($\hat{\sigma}_0^2$) and true rate of change ($\hat{\sigma}_1^2$) when controlling for physical restraint is computed to be -32.33 (using formula: $\hat{\sigma}_{01} = corr(\hat{\sigma}_0^2, \hat{\sigma}_1^2)\sqrt{\hat{\sigma}_0^2 \times \hat{\sigma}_1^2} = -1\sqrt{33.35 \times 31.34} = -32.33$).

In the next section, we will look at unconditional growth models for all mice (complete data set) when physical restraint (Stress) is used as a predictor of both initial status and change.

4.5   Unconditional growth models with uncontrolled effects of Stress (Model C) on complete data set

The multilevel model used in this section for the complete data set will have the same structure as given by Equation 25. This will help us analyze whether the intercept and slope (for level-1 variable 'days') is affected by stress on the complete mouse data set.

The code executed to fit this model using *nlme* package in R is given as:

```
ModelL8 = lme(glucose ~ day * stress, random = ~ day|mouse, data=mice,
na.action = na.exclude)
summary(ModelL8)
```

Similarly, the code executed to fit this model using *lme4* package in R is given as:

$ModelL9 = lmer(glucose \sim day\ *stress + (day\ |\ mouse),\ data=mice,\ na.action$

$= na.exclude)$

$summary(ModelL9)$

Likewise, the SAS code executed to fit this model is given in Table 39.

Table 39: SAS code for model using Stress as level-2 variable and 'Day' as level-1 variable

```
proc mixed data = mice covtest noclprint;
   class mouse;
   model glucose =  day stress day*stress/ solution;
   random day / subject = mouse;
run;
```

The results of the fitted models are given in Tables 40, 41 and 42 respectively.

Table 40: Result for model using Stress as level-2 variable and 'Day' as level-1 variable in *nlme* package on complete mice data set.

```
Linear mixed-effects model fit by REML
 Data: mice1
       AIC      BIC    logLik
  5379.892 5414.105 -2681.946
Random effects:
 Formula: ~day | mouse
 Structure: General positive-definite, Log-Cholesky parametrization
            StdDev       Corr
(Intercept) 12.736417    (Intr)
day          5.402035    -0.66
Residual    29.865808
Fixed effects: glucose ~ day * stress
               Value    Std.Error    DF     t-value   p-value
(Intercept) 115.89661    4.037605    474   28.704294   0.0000
day           3.85154    1.032780    474    3.729299   0.0002
stress        3.05805    5.711105     58    0.535457   0.5944
day:stress   -2.44326    1.460837    474   -1.672506   0.0951
 Correlation:
          (Intr)      day       stress
day        -0.565
stress     -0.707     0.399
day:stress  0.399    -0.707    -0.565
Standardized Within-Group Residuals:
       Min           Q1           Med           Q3          Max
  -4.24398759   -0.51543905   -0.02899827    0.35146256   6.00454848
Number of Observations: 536
Number of Groups: 60
```

Table 41: Result for model using Stress as level-2 variable and 'Day' as level-1 variable in *lme4* package on complete mice data set.

```
Linear mixed model fit by REML ['lmerMod']
Formula: glucose ~ day * stress + (day | mouse)
   Data: mice
REML criterion at convergence: 5363.9
Scaled residuals:
    Min      1Q     Median      3Q       Max
-4.2440   -0.5154   -0.0290   0.3515   6.0045
Random effects:
 Groups    Name         Variance   Std.Dev.   Corr
 mouse    (Intercept)  162.22      12.736
          day           29.18       5.402     -0.66
 Residual               891.97      29.866
Number of obs: 536, groups:  mouse, 60
Fixed effects:
            Estimate   Std. Error   t value
(Intercept)  115.897      4.038      28.704
day            3.852      1.033       3.729
stress         3.058      5.711       0.535
day:stress    -2.443      1.461      -1.673
Correlation of Fixed Effects:
          (Intr)     day      stress
day       -0.565
stress    -0.707    0.399
day:stress 0.399   -0.707    -0.565
```

Also, an AIC value of 5379.892 is generated for *ModelL9* using the codes given below:

```
aic2 = AIC(logLik(ModelL9))

aic2
```

The SAS output result is given in Table 42.

Table 42: Result for model using Stress as level-2 variable and 'Day' as level-1 variable in SAS on complete mice data set.

```
              Covariance Parameter Estimates
Cov Parm     Subject    Estimate    StandError    Z Value     Pr > Z
day           mouse      23.1380      4.4868        5.16      <.0001
Residual                 945.75      61.4683       15.39      <.0001
              Fit Statistics
-2 Res Log Likelihood          5374
AIC (Smaller is Better)        5378
AICC (Smaller is Better)       5378.1
BIC (Smaller is Better)        5382.2
              Solution for Fixed Effects
Effect       Estimate    StandError     DF      t Value     Pr > |t|
Intercept    115.92       3.3988       474      34.11       <.0001
day           3.8533      0.9332        58       4.13       0.0001
stress        3.0144      4.8079       474       0.63       0.5310
day*stress   -2.4414      1.3200       474      -1.85       0.0650
```

Comparing the outputs obtained in Tables 40, 41 and 42, we observe that there is some difference between the SAS and R output results. But, for the most part, the outputs are very close to each other. The fixed and random effects estimates are about the same for all three output results. So, substituting the $\hat{\gamma}$ parameter estimates obtained in tables in the level-2 sub-model given in Equation 23, we can write:

$$\hat{\pi}_{0i} = 115.90 + 3.06\widehat{Stress}_i$$

$$\hat{\pi}_{1i} = 3.85 - 2.44\widehat{Stress}_i$$

(28)

We can then write the composite model as:

$$\widehat{Glucose}_{ij} = 115.90 + 3.06\widehat{Stress}_i + 3.85\widehat{Day}_{ij} - 2.44\widehat{Stress}_i\widehat{Day}_{ij} \qquad (29)$$

The first part of Equation 28 given by $\hat{\pi}_{0i}$ represents the effects of level-2 variable Stress when Day_{ij} is 0 while the second part of Equation 28 given by $\hat{\pi}_{1i}$ represents the effects that level-2 variable Stress has on the slope of level-1 variable Day_{ij}.

Now, interpreting the $\hat{\gamma}$ parameter estimates given in Equation 28, we can say that the average glucose level for all mice at initial status (Day 0) when no physical restraint is given to them (Stress $= 0$) is 115.90. This is also the average glucose level for mice in groups 1, 2 and 3 since all mice in groups 1, 2 and 3 receive no physical restraint. However, for mice that receive physical restraint (Stress $= 1$), the average glucose level is estimated to be 118.96 which is about 3.06 higher than that for mice that do not receive physical restraint. This is also the average glucose level for mice in groups 4, 5 and 6 since only group 4, 5 and 6 mice receive physical restraint.

In this part, we will analyze the second part of Equation 28 given by $\hat{\pi}_{1i}$. This part gives the coefficients for the rates of change. Based on the coefficients given, we can say that the average rate of change in glucose level for mice that do not receive any physical restraint (mice in groups 1, 2 and 3 such that Stress $= 0$) is 3.85. However, the average rate of change in glucose level for mice that receive physical restraint (mice in groups 4, 5 and 6 such that Stress $= 1$) is estimated to be lowered by 2.44 points (at 1.41). Hence, we can say that, on the next day that the glucose level is measured, for mice that do not receive physical restraint, average glucose level

goes up by 3.85 points while for mice that receive physical restraint, average glucose level goes up by only 1.41 points. This suggests that imposing physical restraint on mice decreases their blood glucose level. Figure 8, which represents the results of the fitted multilevel model for change, supports the numerical interpretation. Even though the trajectory for mice that do not face any physical restraint (Stress = 0) is incomplete because of missing data, we can interpolate that this trajectory shows an increasing trend and the average glucose level is much higher for mice that do not experience physical restraint. This plot also shows that for mice that do not experience any physical restraint, the average glucose level is around 115 while for mice that experience physical restraint, the average glucose level is around 118.
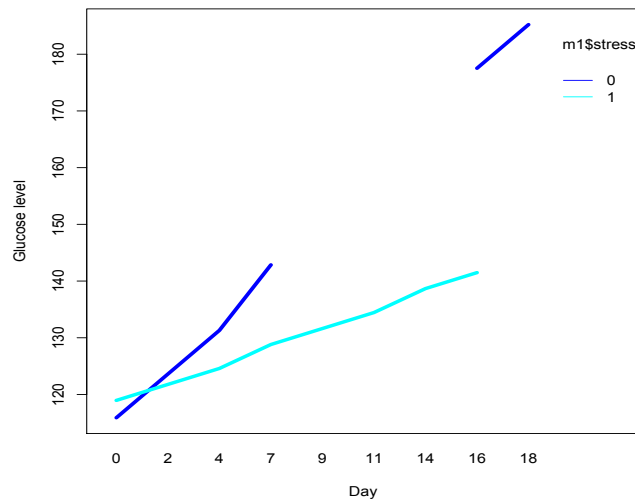

Figure 8: Graphical representation of a fitted multilevel model given in Equation 24 for complete mice data set

Now, we will analyze the variance components or the random effects variances obtained in Tables 40, 41 and 42. Variance components give us the amount of outcome

variability that are unexplained after fitting the multilevel model given in Equation 28. Based on the results obtained, $\hat{\sigma}_\epsilon^2$, which gives the variability in an average mouse's outcome around it's own true change trajectory, is 891.97. SAS gives a higher variance value of 945.75. This is also known as the level-1 residual variance. Similarly, the level-2 variance components as given in Equation 28 as $\begin{bmatrix} \hat{\sigma}_0^2 & \hat{\sigma}_{01} \\ \hat{\sigma}_{10} & \hat{\sigma}_1^2 \end{bmatrix}$ is given by $\begin{bmatrix} 162.22 & -45.41 \\ -45.41 & 29.18 \end{bmatrix}$. So, $\hat{\sigma}_0^2$, which gives the population residual variability of the true initial status (i.e. average intercept when $Day_{ij} = 0$) when controlling for physical restraint (Stress $= 0$ or 1), is about 162.22. Similarly, $\hat{\sigma}_1^2$, which gives the population residual variance of true rate of change (i.e. slope for Day_{ij}) when controlling for physical restraint (Stress $= 0$ or 1), is 29.18. And $\hat{\sigma}_{01}$ which gives the population residual covariance between true initial status ($\hat{\sigma}_0^2$) and true rate of change ($\hat{\sigma}_1^2$) when controlling for physical restraint is computed to be -32.33 (using formula: $\hat{\sigma}_{01} = corr(\hat{\sigma}_0^2, \hat{\sigma}_1^2)\sqrt{\hat{\sigma}_0^2 \times \hat{\sigma}_1^2} = -0.66\sqrt{162.22 \times 29.18} = $ -45.41).

When we compare these variance components to the values of the variance components obtained in section 4.4 (when only considering group 3 and group 6 mice), we notice that the variance components obtained for the complete mice data set is much higher. This could be as a result of the varying doses of STZ that mice in both categories of physical restraint (Stress $= 0$ or 1) receive. Also, comparing the AIC values for models in section 4.4 (group 3 and group 6 data set) and section 4.5 (complete mice data set), we notice that the AIC value is much smaller (1849.085) for the model in section 4.4. The AIC value for the model in section 4.5 is 5379.89. The fact that all mice used in section 4.4 are given the same dose of STZ may explain the lower variance component and lower AIC values. To see if this is the case, in the

next section, we will include variable 'Day' as level-1 predictor variable and variables STZ and Stress as level-2 predictor variables on the complete mice data set.

4.6 Unconditional growth models with uncontrolled effects of Stress and STZ (Model D) on complete data set

This model predicts glucose level based on the intercept and variable 'Day'. It also asks whether the intercept and slope for variable 'Day' are affected by physical restraint (variable name Stress) imposed on the mouse and also by the dose of diabetes inducing drug (variable name STZ) received by the mouse. The structure of this model looks like:

$$\text{Level } 1 : Y_{ij} = \pi_{0i} + \pi_{1i} Day_{ij} + \varepsilon_{ij}$$

$$\text{Level } 2 : \pi_{0i} = \gamma_{00} + \gamma_{01} \times Stress_i + \gamma_{02} \times STZ_i + \zeta_{0i} \tag{30}$$

$$\pi_{1i} = \gamma_{10} + \gamma_{11} \times Stress_i + \gamma_{12} \times STZ_i + \zeta_{1i}$$

where, $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$ and $\begin{bmatrix} \zeta_{0i} \\ \zeta_{1i} \end{bmatrix} \sim \text{N}(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \sigma_0^2 & \sigma_{01} \\ \sigma_{10} & \sigma_1^2 \end{bmatrix})$.

This model evaluates the effects of variable Stress on initial status (when Day_{ij} = 0) and rates of change in glucose level Y_{ij} while keeping the effects of variable STZ on initial status and rate of change constant. Also, γ_{01} and γ_{11} describe the change in glucose level between mice that experience physical restraint (Stress = 1) and mice that do not experience physical restraint (Stress = 0) while keeping the effect of the variable STZ constant. Similarly, γ_{02} and γ_{12} describe the change in glucose level

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between mice that receive 0, 25 or 50 mg kg^{-1} of STZ.

The code executed to fit this model using *nlme* package in R is given as:

$ModelL10 = lme(glucose \sim day * stress + day * STZ, random = \sim day|mouse,$

$data=mice,\ na.action=na.exclude)$

$summary(ModelL10)$

Also, the code executed to fit this model using *lme4* package in R is given as:

$ModelL11 = lmer(glucose \sim day * stress + day * STZ + (day \mid mouse),$

$data=mice,\ na.action=na.exclude)$

$summary(ModelL11)$

Likewise, the SAS code executed to fit this model is given in Table 43.

Table 43: SAS code for model using Stress as level-2 variable and 'Day' as level-1 variable

```
proc mixed data = mice covtest noclprint;
   class mouse;
   model glucose =  day stress STZ day*stress day*STZ/ solution;
   random day / subject = mouse;
run;
```

The results of the fitted models are given in Tables 44, 45 and 46 respectively.

Table 44: Result for model that includes Stress and STZ as level-2 variable and 'Day' as level-1 variable using *nlme* package on the complete mice data set.

```
Linear mixed-effects model fit by REML
 Data: mice
       AIC        BIC        logLik
   5364.198   5406.927   -2672.099
Random effects:
 Formula: ~day | mouse
 Structure: General positive-definite, Log-Cholesky parametrization
            StdDev         Corr
(Intercept) 11.385070    (Intr)
day          4.193854    -0.508
Residual    29.867860
Fixed effects: glucose ~ day * stress + day * STZ
              Value       Std.Error   DF     t-value     p-value
(Intercept) 123.61266    5.159101    473    23.960117   0.0000
day          -0.30766    1.091015    473    -0.281997   0.7781
stress        3.09456    5.517817     57     0.560830   0.5771
STZ          -0.30873    0.135212     57    -2.283296   0.0262
day:stress   -2.44849    1.166683    473    -2.098677   0.0364
day:STZ       0.16631    0.028586    473     5.817776   0.0000
 Correlation:
          (Intr)    day      stress     STZ      day:stress
day       -0.513
stress    -0.533    0.273
STZ       -0.654    0.336    -0.002
day:stress 0.273   -0.533    -0.513     0.001
day:STZ    0.336   -0.655     0.001    -0.513      -0.001
Standardized Within-Group Residuals:
      Min           Q1           Med           Q3           Max
-4.24148011   -0.52208313  -0.03907015    0.37064596   5.96363367
Number of Observations: 536
Number of Groups: 60
```

Table 45: Result for model using Stress and STZ as level-2 variable and 'Day' as level-1 variable in *lme4* package on complete mice data set.

```
Linear mixed model fit by REML ['lmerMod']
Formula: glucose ~ day * stress + day * STZ + (day | mouse)
   Data: mice
REML criterion at convergence: 5344.2
Scaled residuals:
    Min      1Q     Median       3Q        Max
-4.2415  -0.5221  -0.0391    0.3706     5.9636
Random effects:
 Groups   Name         Variance   Std.Dev.    Corr
 mouse    (Intercept)  129.62      11.385
          day           17.59       4.194      -0.51
 Residual              892.09      29.868
Number of obs: 536, groups:  mouse, 60
Fixed effects:
             Estimate    Std. Error     t value
(Intercept)  123.61266     5.15910       23.960
day           -0.30766     1.09102       -0.282
stress         3.09456     5.51781        0.561
STZ           -0.30873     0.13521       -2.283
day:stress    -2.44849     1.16668       -2.099
day:STZ        0.16631     0.02859        5.818
Correlation of Fixed Effects:
          (Intr)     day    stress     STZ    day:stress
day       -0.513
stress    -0.533    0.273
STZ       -0.654    0.336   -0.002
day:stress 0.273   -0.533   -0.513    0.001
day:STZ    0.336   -0.655    0.001   -0.513   -0.001
```

Also, an AIC value of 5364.198 is generated for *ModelL11* using the codes given

below:

```
aic3 = AIC(logLik(ModelL11))

aic3
```

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Table 46: Result for model using Stress and STZ as level-2 variable and 'Day' as level-1 variable in SAS on complete mice data set.

```
                  Covariance Parameter Estimates
Cov Parm     Subject    Estimate    StandError    Z Value     Pr > Z
day           mouse     14.5883      2.9255        4.99 <.0001
Residual                934.60      60.8299       15.36 <.0001
                  Fit Statistics
-2 Res Log Likelihood          5348.9
AIC (Smaller is Better)        5352.9
AICC (Smaller is Better)       5352.9
BIC (Smaller is Better)        5357.1
                  Solution for Fixed Effects
Effect        Estimate     StandError     DF      t Value    Pr > |t|
Intercept      123.61        4.4679       473      27.67       <.0001
day           -0.3070        1.0115        57      -0.30 0.7626
stress         3.0856        4.7794       473       0.65 0.5189
STZ           -0.3085        0.1171       473      -2.63 0.0087
day*stress    -2.4484        1.0817       473      -2.26       0.0241
day*STZ        0.1663        0.0265       473       6.27       <.0001
```

Comparing the outputs obtained in Tables 44, 45 and 46, we observe that the fixed and random effects estimates are the same for both packages in R. Also, the output obtained using SAS is very close to the two R outputs. AIC values for all models are very close to each other. Now, substituting the estimated γ parameters obtained in the tables in the level-2 sub-model given in Equation 30, we can write the new equation as given in Equation 31.

$$\hat{\pi}_{0i} = 123.6127 + 3.0946\widehat{Stress}_i - 0.3087\widehat{STZ}_i$$

$$\hat{\pi}_{1i} = -0.3077 - 2.4485\widehat{Stress}_i + 0.1663\widehat{STZ}_i$$

(31)

We can then write the composite model as:

$$\widehat{Glucose}_{ij} = 123.6127 + 3.0946\widehat{Stress}_i - 0.3087\widehat{STZ}_i - 0.3077\widehat{Day}_{ij}$$
$$-2.4485\widehat{Stress}_i\widehat{Day}_{ij} + 0.1663\widehat{STZ}_i\widehat{Day}_{ij} \tag{32}$$

For model D, since it includes two predictor variables in level-2, the intercepts 123.6127 and -0.3077 describe initial state and rate of change for mice that do not experience physical restraint (Stress $= 0$) and receive 0 mg kg^{-1} STZ. Also, we can conclude that when keeping the effects of STZ constant, the estimated difference in initial glucose level between mice that experience physical restraint and mice that do not experience physical restraint is 3.0946. This difference, with a p-value of 0.5771 is insignificant. We can also conclude that, keeping the effects of STZ constant, the estimated difference in the rate of change in glucose level between mice that experience physical restraint and mice that do not experience physical restraint is -2.4485. This estimate is significant with a p-value of 0.0364. Similarly, keeping the effects of Stress constant, we can conclude that the estimated difference in initial glucose level between mice that receive 25 mg kg^{-1} and mice that receive 50 mg kg^{-1} is -0.3087. This estimate is significant with a p-value of 0.0262. Also, keeping the effects of Stress constant, the estimated difference in the rate of change in glucose level between mice that receive 25 mg kg^{-1} and mice that receive 50 mg kg^{-1} is 0.1663. This estimate is also significant with a p-value of 0.00.

Now, we will analyze the variance components or the random effects variances obtained in Tables 44, 45 and 46. Variance components give us the amount of outcome

variability that are unexplained after fitting the multilevel model given in Equation 32. Based on the results obtained, $\hat{\sigma}_\epsilon^2$, which gives the variability in an average mouse's glucose level around its own true change trajectory, is 892.09. This is also known as the level-1 residual variance. Similarly, the level-2 variance components as given in Equation 23 as $\begin{bmatrix} \hat{\sigma}_0^2 & \hat{\sigma}_{01} \\ \hat{\sigma}_{10} & \hat{\sigma}_1^2 \end{bmatrix}$ is given by $\begin{bmatrix} 129.62 & -24.30 \\ -24.30 & 17.59 \end{bmatrix}$. So, $\hat{\sigma}_0^2$, which gives the population residual variability of the true initial status (i.e. average intercept when $Day_{ij} = 0$) when controlling for physical restraint (Stress =0 or 1) and amount of STZ, is about 129.62. Similarly, $\hat{\sigma}_1^2$, which gives the population residual variance of true rate of change (i.e. slope for Day_{ij}) when controlling for physical restraint (Stress =0 or 1) and STZ, is 17.59. And $\hat{\sigma}_{01}$ which gives the population residual covariance between true initial status $(\hat{\sigma}_0^2)$ and true rate of change $(\hat{\sigma}_1^2)$ when controlling for both physical restraint and STZ is computed to be -24.30 (using formula: $\hat{\sigma}_{01} = corr(\hat{\sigma}_0^2, \hat{\sigma}_1^2)\sqrt{\hat{\sigma}_0^2 \times \hat{\sigma}_1^2} = -0.51\sqrt{129.09 \times 17.59} =$ -24.30).

When we compare these variance components to the values of the variance components obtained in section 4.5 (when only Stress is used as a level-2 predictor variable), we notice that the variance components obtained for in this section for model D is smaller. This could be as a result of including variable STZ as a level-2 predictor variable which helps explain some variability in glucose level between mice. Also, comparing the AIC values for models in section 4.5 (Model C on complete mice data set) and section 4.6 (Model D on complete mice data set), we notice that the AIC value is smaller (5364.198) for the model in section 4.6. The AIC value for the model in section 4.5 is 5379.89. AIC and BIC scores are very similar among the two packages used in R and SAS.

5 FURTHER EXPLORATION OF INDIVIDUAL MODELS

In previous sections we have discussed and compared existing methodologies for analysis of longitudinal data. In this section we will explore a different path: to fit individual regression models to the data for each individual and then conduct analyses on the estimated parameters.

5.1 Linear Models

In this section we will fit separate linear regression models for all mice using 'Day' as an explanatory variable. The code used to fit these model uses the *lme4* package and can written as:

```
a = lmList(glucose ∼ day|mouse, data = mice)

summary(a)
```

Since there are 60 different mice, 10 mice in each group, the above code gives us 60 different linear models. In order to compare the intercepts and slopes obtained for each mice in each group, we will create dot plots for the slopes and intercepts in each linear model as in Figures 9 and 10. In both plots, the solid (filled black) circles represent the group's average slope or average intercept values.
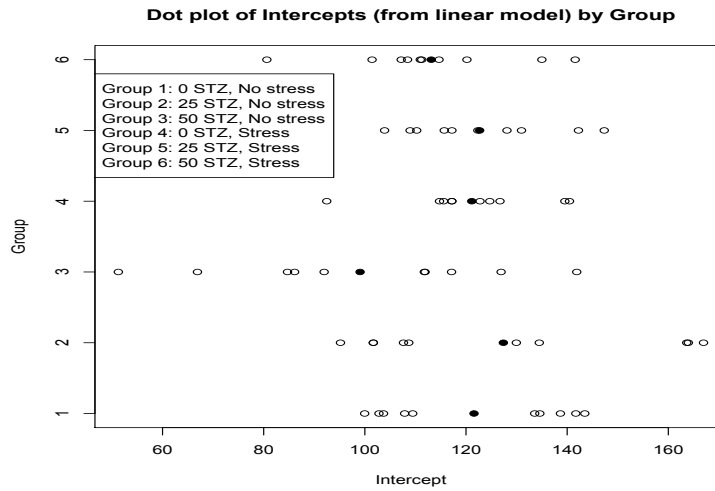
Figure 9: Dot plot for the intercepts of linear models fitted for each of the 60 mice, separated by groups

Figure 10: Dot plot for the slope of the linear models fitted for each of the 60 mice, separated by groups

5.2 Levene's Test

Observing the dot plots, we notice greater variability in intercept and slope for mice in groups 3 and 6. These are the groups of mice that receive 50 mg kg^{-1} of STZ with and without physical restraint respectively. A higher dose of STZ could be associated with the large variability in slope and intercepts. Since there seems to be unequal variances among the different groups for both slope and intercept, we will perform Levene's test to check if this is actually the case. The hypothesis for the Levene's test can be written as:

$$H_0 : \sigma_1^2 = \sigma_2^2 = \sigma_3^2 = \sigma_4^2 = \sigma_5^2 = \sigma_6^2$$

$$H_a : \text{At least one } \sigma_i^2 \text{ is different, i} = 1, 2,.., 6$$

The code executed to perform Levene's test in R can be written as:

```
leveneTest(variable_name, group_variablename)

leveneTest(int_estimate, group)

leveneTest(day_estimate,group)
```

The result for Levene's test performed to see if the variances in 'intercept' and 'slope' are same among the different groups of mice are given in Tables 47 and 48 respectively .

Table 47: Result for Levene's test performed to check equal variance of 'intercept' among the 6 groups of mice.

```
Levene's Test for Homogeneity of Variance (center = median)
      Df F value  Pr(>F)
group  5  3.1833 0.01368 *
      54
```

Table 48: Result for Levene's test performed to check equal variance of 'slope' among the 6 groups of mice.

```
Levene's Test for Homogeneity of Variance (center = median)
      Df F value    Pr(>F)
group  5  4.6666 0.001299 **
      54
```

For both 'intercept' and 'slope', the *p-values* are significant (0.0137 and 0.0013), therefore, we have enough evidence to say that at least one of the groups of mice have different variance for both 'intercept' and 'slope'.

5.3   One-Way ANOVA (assuming unequal variance)

In this section, we will perform One-Way ANOVA on the two variables 'slope' and 'intercept' assuming unequal variances. The hypothesis for One-Way ANOVA can be written as:

$$H_0 : \mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6$$

$$H_a : \text{At least one } \mu_i \text{ is different, i} = 1, 2,.., 6$$

The R code used to perform One-Way ANOVA on variable 'intercept' assuming unequal variances is given as:

oneway.test(int_estimate ∼ group, b,na.action=na.exclude, var.equal = FALSE)

The output obtained for the analysis is given below in Table 49.

Table 49: One-way ANOVA for 'intercept' assuming unequal variances

```
 One-way analysis of means (not assuming equal variances)
data:  int_estimate and group
F = 1.4932, num df = 5.000, denom df = 24.957, p-value = 0.2277
```

Since the *p-value* is not small (0.2277), we do not have enough evidence to conclude that the mean intercept value is different among the six groups.

Similarly, the R code used to perform One-Way ANOVA on variable 'slope' assuming unequal variances is given as:

$oneway.test(day\_estimate \sim group, b, na.action=na.exclude, var.equal = FALSE)$

The output obtained for the analysis is given below in Table 50.

Table 50: One-way ANOVA for 'slope' assuming unequal variances

```
One-way analysis of means (not assuming equal variances)
data:  day_estimate and group
F = 7.9409, num df = 5.000, denom df = 24.691, p-value = 0.0001418
```

Since the *p-value* is very small (0.0001), we have enough evidence to conclude that the mean slope is different for at least one group. This suggests that the mean slope is not the same across all groups, i.e. at least one group is different from the others.

5.4 Two-Way ANOVA (assuming equal variances)

In the previous section we compared the six groups considering them as six different treatments. Now we will refine the analysis a little more taking into consideration that those six treatments are actually the combination of the levels of the two factors. We will fit four Two-Way ANOVA models. Later we will also fit ANOVA models assuming unequal variances. The first model will assume the intercept to be the response variable while treating the variables Stress and STZ as the two factors. The second model will include an interaction between the two factors. Similarly, the third model will assume the slope to be the response variable while treating variables Stress and STZ as the two factors. And the fourth model will include an interaction between the two factors for the new response variable.

By fitting these models, we are trying to analyze if the amount of STZ has an effect on the intercept and slope. We also want to know if physical restraint experienced by a mice (Stress) has an effect on the intercept and slope. Also, we want to know if there is an interaction between the STZ and Stress. While, we attempt to answer these questions, let us first look at some side-by-side box plots to get a clear picture of how STZ and Stress impact 'slope' and 'intercept' of linear models for all mice.
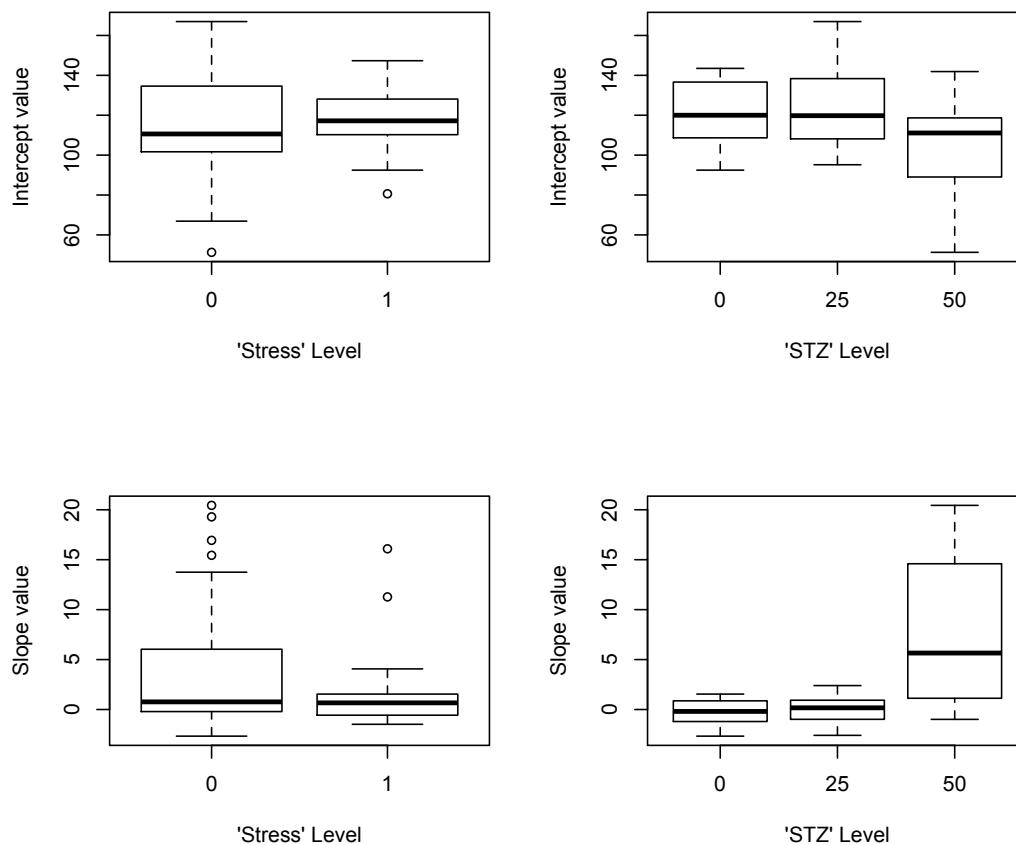
Figure 11: Box plots for slopes and intercepts separated by factor levels

Observing the box plots, we notice that there exists some difference in the response variable 'intercept' for the two different levels of Stress. The variability in intercept shown by mice that experience physical restraint (Stress = 1) is smaller than the variability shown by mice that do not experience physical restraint (Stress = 0). However, looking at the effect of STZ, the effect of 50 mg kg^{-1} of STZ on mice seems to be much more distinguished than the effect that is shown by the mice that are given the two other levels of STZ(0 mg kg^{-1} or 25 mg kg^{-1}). This trend is also true

for response variable 'slope'. Also, observing the interaction plot given in Figure 12, we notice that some kind of interaction seem to be present between Stress and STZ since the lines are not all parallel to each other. Also, it is evident that this interaction is much distinguished for 50 mg kg^{-1} of STZ for both response variables. Now, in order to find out if these factors and interaction between factors are significant, let us start by fitting the different models.
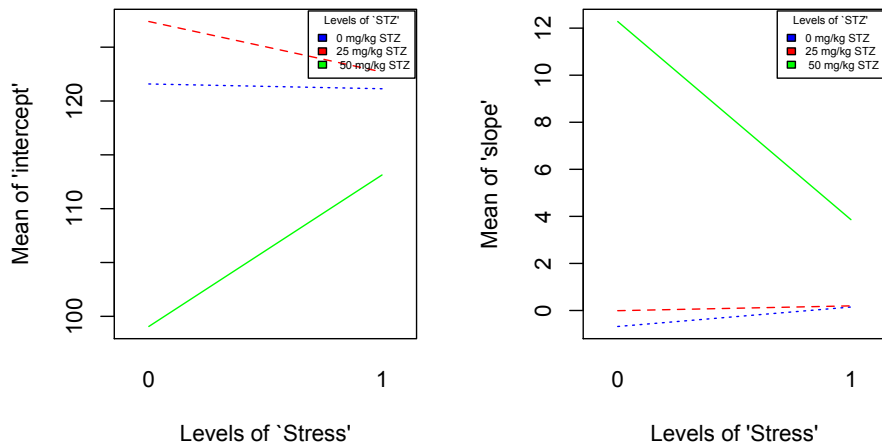

Figure 12: Interaction plot between Stress and STZ for 'slope' and 'intercept'

We will fit ANOVA models for the intercept and the slope as response variables but the focus of the interpretation should be on the slope that represents the change in glucose level through time. In order to fit these models we will use the *lm()* function in R. The generic code to fit a two-way ANOVA model without interaction can be written as:

lm(Response ∼ FactorA + FactorB)

Similarly, the generic code to fit a two-way ANOVA model with interaction can

be written as:

lm(Response ∼ FactorA + FactorB + FactorA*FactorB)

Now, we will fit the first Two-way ANOVA model (*M1*) without interaction considering the 'intercept' to be the response variable. The code to fit this model can be written as:

M1 = lm(intercept ∼ Stress + STZ)

summary(M1)

The results of model *M1* is given in Table 51.

Table 51: Result of ANOVA fitted with 'intercept' as response variable and Stress and STZ as two factors.

```
Call:
lm(formula = int_estimate ~ stress + stz)
Residuals:
     Min       1Q     Median      3Q       Max
  -57.112   -14.214   -1.849    13.149    50.951
Coefficients:
            Estimate   Std. Error   t value    Pr(>|t|)
(Intercept) 123.6412     5.1749     23.892     <2e-16 ***
stress        2.9806     5.5322      0.539     0.5921
stz          -0.3053     0.1355     -2.253     0.0281 *
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
Residual standard error: 21.43 on 57 degrees of freedom
Multiple R-squared:  0.08605,      Adjusted R-squared:  0.05398
F-statistic: 2.683 on 2 and 57 DF,  p-value: 0.07697
```

Observing the output of the ANOVA table for model *M1*, we notice that the main effect of Stress is not significant.Therefore, we can say that the observed difference in 'intercept' between the two different levels of Stress is not statistically significant.

However, the main effect of STZ shows significant effect on the 'intercept' with a *p-value* of 0.0281. So, we can say that the observed difference in 'intercept' between the three different levels of STZ is statistically significant.

Now, we will fit the second Two-way ANOVA model (*M2*) that includes interaction between the two factors Stress and STZ. The code to fit this model can be written as:

M2 = lm(intercept ∼ Stress + STZ + Stress*STZ)

summary(M2)

The results obtained from fitting model *M2* is given in Table 52.

Table 52: Result of ANOVA fitted with 'intercept' as response variable and Stress and STZ as two factors. This model also includes an interaction term between Stress and STZ.

```
Call:
lm(formula = int_estimate ~ stress + stz + stress * stz)
Residuals:
    Min       1Q     Median      3Q       Max
 -53.482   -13.751   -2.533     12.783    50.951
Coefficients:
            Estimate   Std. Error   t value    Pr(>|t|)
(Intercept) 127.2708      6.1771     20.604     <2e-16 ***
stress       -4.2785      8.7357     -0.490     0.6262
stz          -0.4505      0.1914     -2.354     0.0221 *
stress:stz    0.2904      0.2707      1.073     0.2880
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
Residual standard error: 21.4 on 56 degrees of freedom
Multiple R-squared:  0.1045,      Adjusted R-squared:  0.05648
F-statistic: 2.177 on 3 and 56 DF,  p-value: 0.1008
```

Observing the output of the ANOVA model *M2* in Table 52, we notice that the

interaction between the two factors Stress and STZ on the response variable 'intercept' is not significant. Also, we notice that the main effect of Stress is still not significant in model *M2*. However, the main effect of STZ shows significant effect on the 'intercept' with a *p-value* of 0.0221.

Now, we will fit a third Two-way ANOVA model (*M3*) without interaction considering 'slope' to be the response variable. The code to fit this model can be written as:

$M3 = lm(slope \sim Stress + STZ)$

$summary(M3)$

The results obtained by fitting model *M3* is given in Table 53.

Table 53: Result of ANOVA fitted with 'slope' as response variable and Stress and STZ as two factors.

```
Call:
lm(formula = day_estimate ~ stress + stz)
Residuals:
   Min      1Q    Median      3Q       Max
 -7.188   -2.827   -1.294    2.278    12.406
Coefficients:
            Estimate    Std. Error    t value     Pr(>|t|)
(Intercept) -0.30400      1.09513     -0.278       0.7823
stress      -2.46325      1.17074     -2.104       0.0398 *
stz          0.16675      0.02868      5.815       2.89e-07 ***
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
Residual standard error: 4.534 on 57 degrees of freedom
Multiple R-squared:  0.4015,      Adjusted R-squared:  0.3805
F-statistic: 19.12 on 2 and 57 DF,  p-value: 4.43e-07
```

Observing the output of the ANOVA model *M3* in Table 53, we notice that the

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main effect of Stress is significant on the 'slope' with a *p-value* of 0.0398. Therefore, we can say that the observed difference in 'slope' between the two different levels of Stress is statistically significant. Similarly, the main effect of STZ also shows significant effect on the 'slope' with a *p-value* of 2.89×10^{-7}. So, we can conclude that the difference observed in 'slope' between the three different levels of STZ is also statistically significant.

Now, we will fit the final Two-way ANOVA model (*M4*) that includes interaction between Stress and STZ considering 'slope' to be the response variable. The code to fit this model can be written as:

M4 = lm(slope ~ Stress + STZ + Stress*STZ)

summary(M4)

The results obtained by fitting model *M4* is given in Table 54.

Table 54: Result of ANOVA fitted with 'slope' as response variable and Stress and STZ as two factors. This model also includes an interaction term between Stress and STZ.

```
Call:
lm(formula = day_estimate ~ stress + stz + stress * stz)
Residuals:
    Min       1Q     Median      3Q       Max
 -9.4996   -2.8268   -0.0807   1.8459    12.8330
Coefficients:
            Estimate   Std. Error   t value    Pr(>|t|)
(Intercept) -2.61602    1.19403     -2.191     0.032636 *
stress       2.16080    1.68861      1.280     0.205951
stz          0.25923    0.03700      7.007     3.37e-09 ***
stress:stz  -0.18496    0.05232     -3.535     0.000826 ***
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
Residual standard error: 4.136 on 56 degrees of freedom
Multiple R-squared:  0.5107,        Adjusted R-squared:  0.4845
F-statistic: 19.48 on 3 and 56 DF,  p-value: 8.934e-09
```

Observing the output of the ANOVA model *M2* in Table 54, we notice that the interaction between the two factors Stress and STZ on the response variable 'slope' is significant with a *p-value* is 0.000826. Therefore, we can say that an interaction between Stress and STZ exists. Also, we notice that the main effect of Stress is not significant in model *M4*. However, the main effect of STZ shows significant effect on the 'slope' with a *p-value* of 3.37×10^{-9}. We can compare these results with that of PROC MIXED on Page 25 and MANOVA on Pages 27 and 28. In the PROC MIXED analysis the interaction and each factor all had a significant effect on glucose. MANOVA indicated that only STZ had a significant effect on glucose. However, in the method developed in this section what we analyze is the effect of STZ and Stress on the way that glucose grows through time.

5.5 Kruskal-Wallis Test

Now, we will apply the Kruskal-Wallis test to see if the samples for the different treatments come the same population. The hypothesis for this test can be written as:

H_0: The samples for the treatments come from the same population

H_1 : The samples for the treatments do not come from the same population

The R code used to perform the test on the intercept can be written as:

$kruskal.test(int\_estimate \sim group, \ data = b)$

The output result of the test performed on the intercept is given in Table 55. Since the *p-value* obtained for the Kruskal-Wallis test is 0.2615, we do not have enough evidence to say that the samples for the treatment intercepts do not come from the sample population. It is actually the slope that we care the most about since they are indicators of how the glucose grows through time for each individual.

Table 55: Kruskal-Wallis test with 'intercept' as response and 'group' as predictor variable

```
        Kruskal-Wallis rank sum test
data:  int_estimate by group
Kruskal-Wallis chi-squared = 6.4892, df = 5, p-value = 0.2615
```

The R code used to perform the test on the slope can be written as:

$kruskal.test(day\_estimate \sim group, \ data = b)$

The output result of the test performed on the slope is given in Table 56. Since, the *p-value* obtained for the Kruskal-Wallis test is 6.215×10^{-5}, we have enough evidence to say that the samples for the treatment slopes do not come from the same population.

Table 56: Kruskal-Wallis test with 'slope' as response and 'group' as predictor variable

```
        Kruskal-Wallis rank sum test
data:  day_estimate by group
Kruskal-Wallis chi-squared = 26.809, df = 5, p-value = 6.215e-05
```

5.6 Randomization Test

Another way of comparing the slopes would be using the randomization test. The randomization test is also known as permutation test. When performing the randomization test, each individual values (in this case the slope for each mouse) is randomly assigned to one of k groups (k = 6 in the example). The mean value $(\bar{y}_k)$ for each random groups is calculated and compared to the general mean $(\bar{y})$ using the statistic $\Sigma(\bar{y}_k - \bar{y})^2$. The value of the statistic is then compared with the one calculated from the original groups defined by the treatments. This process is then repeated multiple times, and a p-value is calculated based on the number of times that the statistic from the random groups is equal or greater than the statistic for the original data. I wrote my own code in R to implement the randomization test. The result of the permutation test is given Figure 13 where the empirical distribution of the values of the statistic are shown. The p-value of this test is 0. The value of the statistic $\Sigma(\bar{y}_k - \bar{y})^2$ (124.67) is denoted by an arrow. Since, the true value is on the extreme right of the

histogram, the *p-value* or the area under the distribution to the right of 124.67 is almost 0. So, we are able to conclude that the observed difference in slopes is not likely to happen just by chance if there is not really any difference among treatments.

Strip chart for SS deviation from overall mean for all groups (Slope)
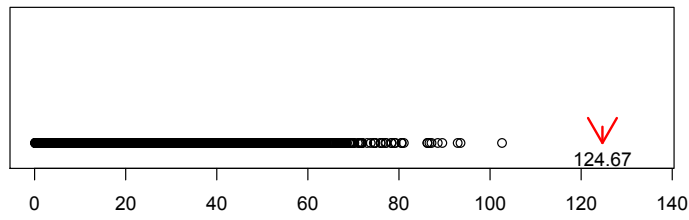
Histogram of SS deviation from overall mean for all groups (Slope)
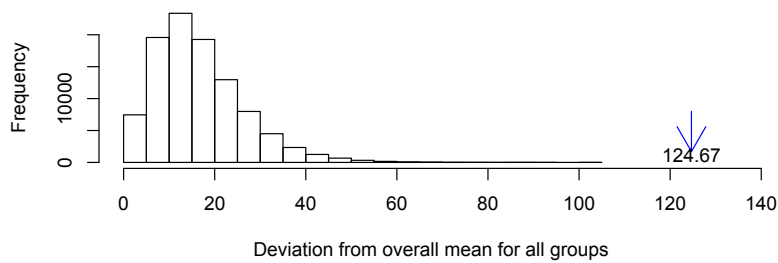

Figure 13: Randomization test performed on the slope of the individual linear regression models

5.7   Power Study

In previous sections we have applied the experimental method to the *mice* example but now we wonder how well this method works to detect differences among groups depending on how the slopes are. In other words we want to explore the power of this test using simulation. We simulated longitudinal data for individuals in three groups and five days using the model:

$$Y_{ijt} = a + b_i t + \varepsilon_{ijt}, \qquad i = 1 : 3; \tag{33}$$

where, the intercepts were randomly generated using the U(0, 2) distribution, $b_i = 1 + (i - 1)d$ for $d = 0.1, 0.2, 0.3, 0.4, 0.5$, t = 1:5 and $\varepsilon_{ijt} \sim N(0, 1)$. The simulation was done for different sample sizes in each group $n_i = 5, 10, 15, 20$. These simulated values are then used to produce a response variable 'Y'. A box plot is presented in Figure 14 for the simulated 'Y' values based on the three groups for one of the simulations. We then use these Y values to fit linear regression models using 'time' as the explanatory variable. One-way ANOVA test is then applied to the slope estimates from the linear models using these estimates as the response variable and 'group' as the factor. The power of the ANOVA test is then analyzed. Figure 17 represents the power of the test for different 'd' values and sample sizes (n = 5, 10, 15, 20). The 'd' values ($d = 0.1, 0.2, ..., 0.5$) are the differences between the slopes of the different groups.
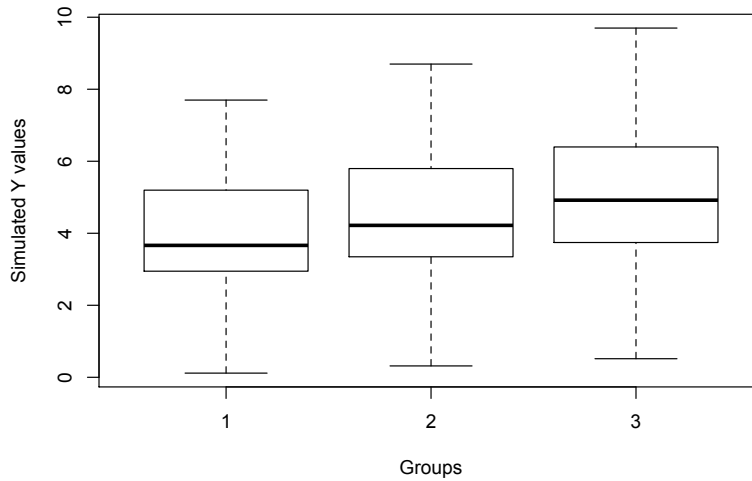
Figure 14: Box plot of the simulated 'Y' values

Figure 15 shows the *p-value* for 100 simulations with $d = 0.4$ but changing the sample size. WE notice that as the sample size increases the mean *p-value* decreases and the whole distribution of *p-values* is concentrated in the really small values.

Figure 16 displays the x values for sample size 15 changing the different d from 0.1 to 0.5. As the difference in slopes increases, the average *p-value* becomes smaller as expected. When the difference in slopes used to generate the data is 0.3, the *p-values* already fall very close to 0.
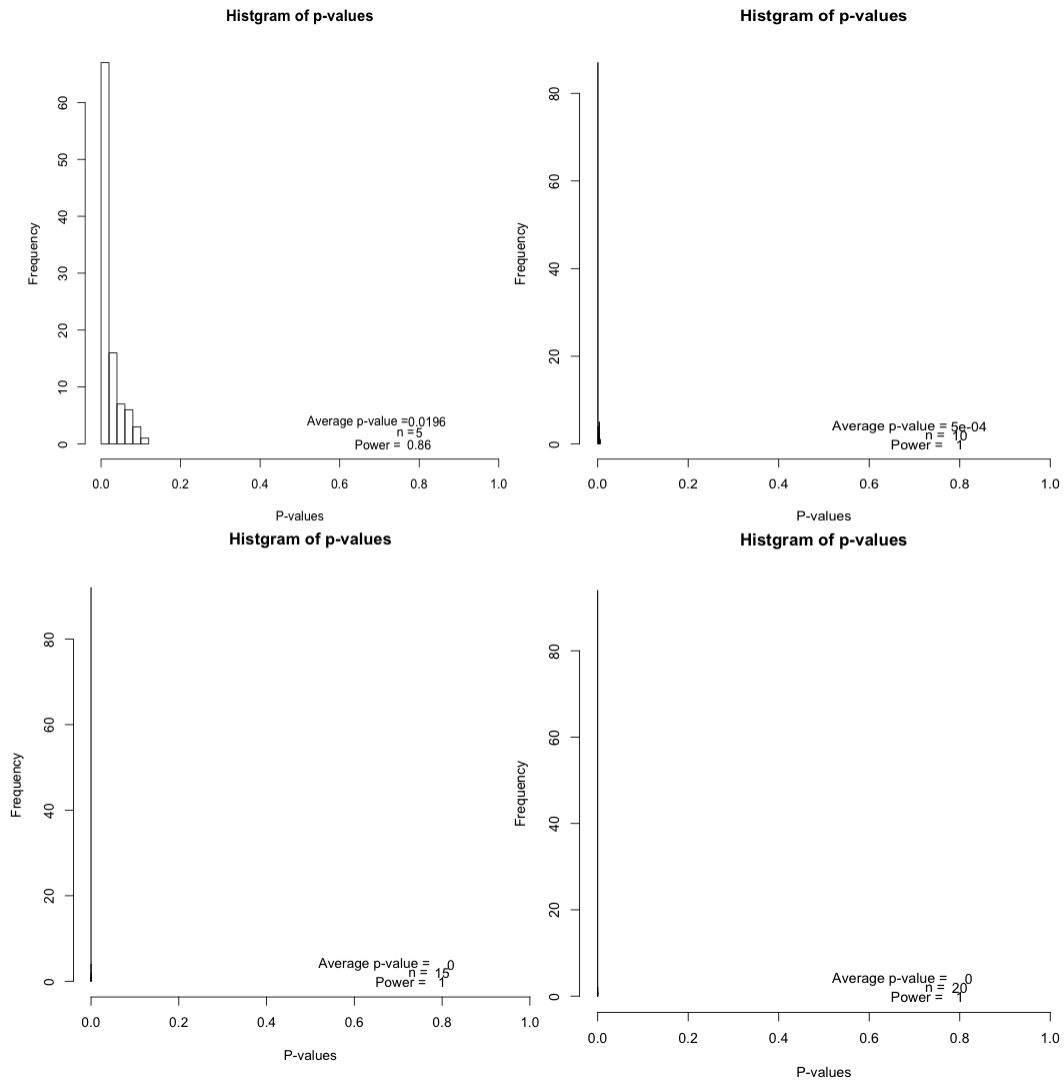
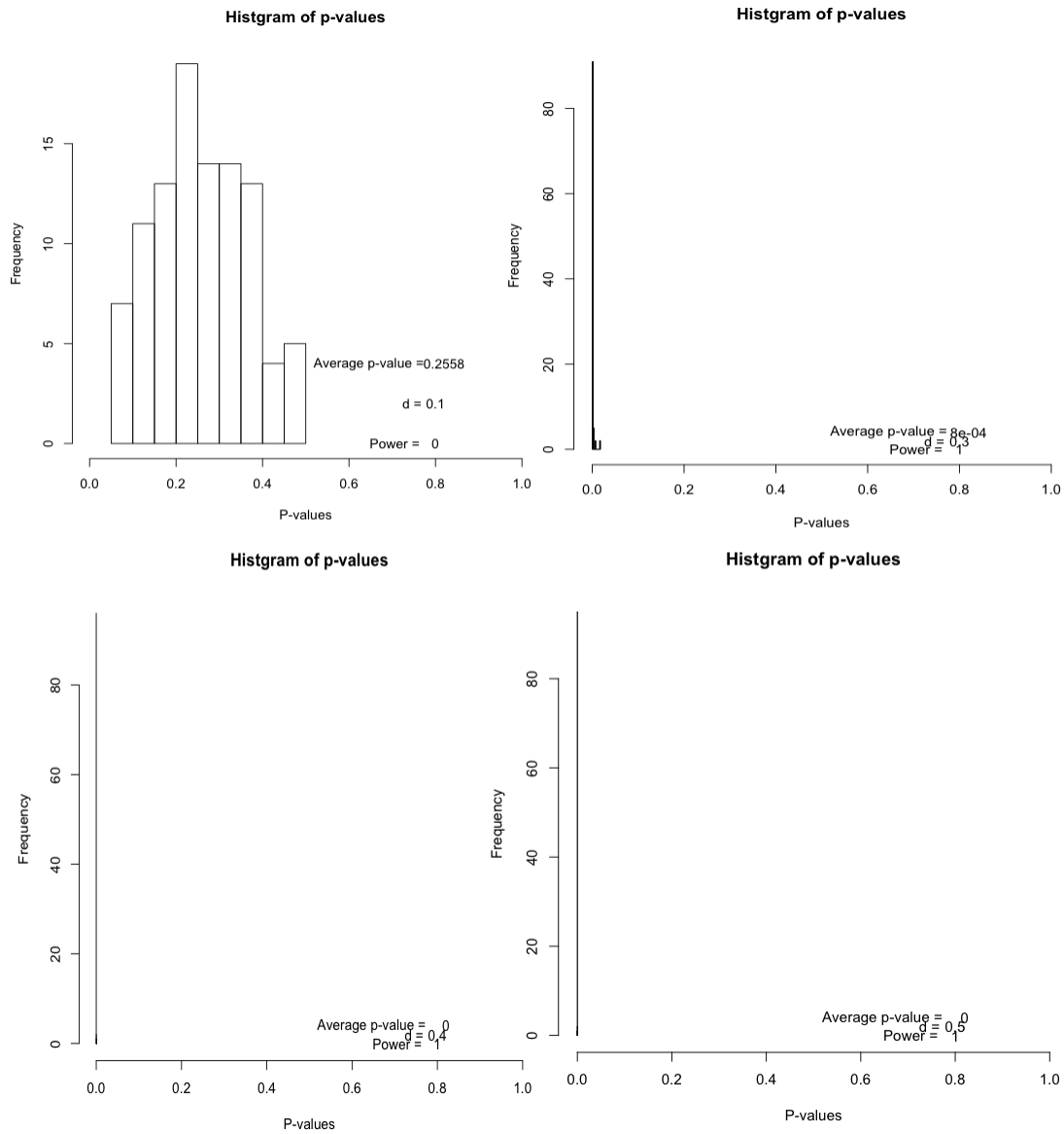Figure 15: Histogram of power for different n values while d is fixed

Figure 16: Histogram of power for different d values while n is fixed

Based on Figures 15, 16 and 17, we notice that the power of the test increases as the numbers of observations (n) included in each group increases. Similarly, power of the test is also higher for larger 'd' values.
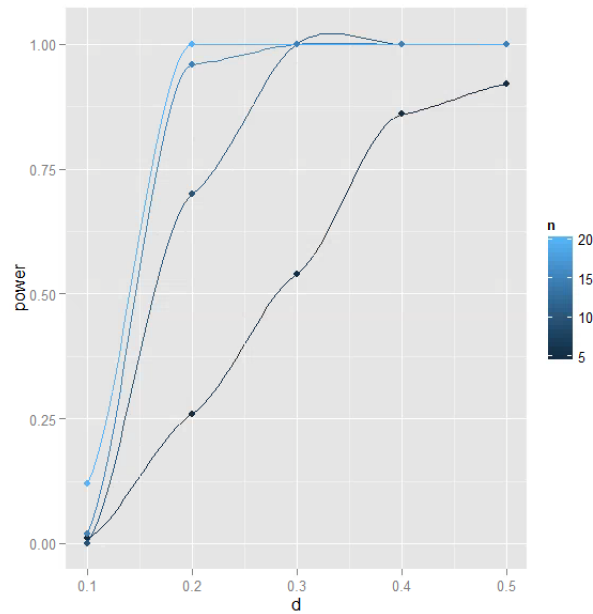
Figure 17: Power test performed on the slope of the simulated linear regression model for different slopes

One advantage of this new method is that each individual is represented by one number: the slope of a linear model fitted to the data for that individual through time. Then the slopes can be analyzed by an ANOVA model dictated by the design of the experiment (one-way, two-way, etc). The power analysis we did has satisfactory results for sample sizes 10 (per group) and higher. One criticism that would be done is that the goodness of the fit of the individual models is not taken into consideration. This could be solved by accompanying the ANOVA of the slopes by an analysis of the R-squared of the regression lines. This is something we plan to include in future research. Another thing left to explore is the study of the power when the growth through time is not linear but we use a linear model to represent it.

Another idea to explore would be to work with slopes for each individual, not estimated using a regression model but estimated from a more non-parametric point

of view. For each individual take increment per time unit for each pair of consecutive measures and then find the mean or the median of such slopes. In that way we are not assuming a rigid linear model. Even more, if more detail was wanted that analysis could be done at each time starting in time $= 2$.

6 CONCLUSION

This thesis focuses on using multilevel models to analyze longitudinal data sets but it also includes a description on the different traditional methods that are commonly used in longitudinal data analysis. Some of the traditional methods over viewed on this thesis is Mixed Effects Models and MANOVA. Also a new method based on the analysis of individual models is proposed. In the next part of the thesis, multilevel models are developed for a case study. The case study involved looking at how acidity of soil at different locations affected the total exchange capacity of the soil. In this case study, six different locations were grouped into three different sites. Five different soil samples were then taken from different locations such that soil samples were nested on locations. When constructing multilevel models for this case study, sample observations were considered to the first level while location was considered to the second level of multilevel models. Several models, including a null model and some random slope coefficient models were fitted for this case study. Based on the analysis performed on the *soildata* case study, we concluded that the explanatory variable which is the pH measure of the soil content is a significant predictor of the response variable which is the total exchange capacity measure of the soil. Similar results were obtained for the two R packages, *nlme* and *lme4*, and SAS.

In the next part of the thesis, we analyzed longitudinal data sets using multilevel models. The data set analyzed in this section was obtained from a case study performed on mice. In this case, we analyzed if change in individual glucose level for mice differed based on two factors - physical restraint imposed on the mice and the

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amount of drug (STZ) given to them. Since mice in one particular group (group 3) showed unusually high glucose level, this fact was considered when developing some models. When fitting models for this case study, Day_{ij} is considered to be the level-1 predictor while STZ_{ij}, $Stress_{ij}$ or the interaction between the two are considered to be the level two predictor variables. The unconditional growth model developed for the group three mice showed the level-1 predictor variable Day_{ij} to be significant. This was also true when the same analysis was performed on all mice (not just group 3).

In an attempt to compare the effect of factor *Stress* and it's interaction with variable Day_{ij} for groups 3 and 6, a model was developed using *Stress* as the level-2 predictor variable, while using Day_{ij} as the level-1 predictor variable. For this model, we were not able to conclude any significant interaction between variables *Day* and *Stress* when the two groups of mice were considered. Result from similar analysis performed on all mice also showed insignificant interaction between variables *Day* and *Stress*. A next model developed using *Stress* and *STZ* as level-2 predictor variables while using *Day* as the level-1 predictor variable showed significant interaction between variables *Day* and *Stress* and variables *Day* and *STZ*.

The output given by multilevel models were somewhat different than that obtained from mixed effects model. While the multilevel models gave us the within and between cluster values, these values were not obtained from the outputs for mixed effects model. Also, the output from multilevel models were comparatively harder to analyze than the output from mixed effects models.

In the last section, a new method to analyze longitudinal data sets is proposed.

In this proposed method, individual linear models are fitted for each individual mice considering time, *Day*, to be the predictor variable. Analysis of variance is then applied to the estimated parameters of the model. This method was able to identify differences between groups for the *mice* example. It also detected the existence of interaction between Stress and STZ and the importance of the effect of STZ when a two-way ANOVA is performed to the estimated slope. A power analysis using simulations gave satisfactory results for sample sizes 10 (per group) and higher. Topics for future research with regard to the new method were identified: to include an analysis of goodness of fit for the individual methods and to explore the power of the test when the thought growth in some groups is not linear.

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