

## Chapter 220

# Mixed Models - General

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## Introduction

The Mixed Models procedure analyzes results from a wide variety of experimental designs in which the outcome (response) is continuous, including

- Two-sample designs (replacing the t-test)
- One-way layout designs (replacing one-way ANOVA)
- Factorial designs (replacing factorial GLM)
- Split-plot designs (replacing split-plot GLM)
- Repeated-measures designs (replacing repeated-measures GLM)
- Cross-over designs (replacing GLM)
- Designs with covariates (replacing GLM)

The Mixed Models procedure can be used to test and estimate means (including pair-wise comparisons among levels), compare models, estimate variance-covariance matrix components, and produce graphs of means and repeated measurements of subjects. Examples are given in this chapter of models with only between-subjects factors, only within-subjects factors, and both between- and within-subjects factors. Analysis of covariance examples and multiple comparisons examples are also included.

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## Why Use a Mixed Model?

As stated above, mixed models have several advantages over traditional linear models. Just a few are listed here.

- **Specifying More Appropriate Variance-Covariance Structures for Longitudinal Data:** The ability to fit complex covariance patterns provides more appropriate fixed effect estimates and standard errors.
- **Analysis Assuming Unequal Group Variances:** Different variances can be fit for each treatment group.
- **Analysis of Longitudinal Data with Unequal Time Points:** Mixed models allow for the analysis of data in which the measurements were made at random (varying) time points.
- **Analysis of Longitudinal Data with Missing Response Data:** Problems caused by missing data in repeated measures and cross-over trials are eliminated.
- **Greater Flexibility in Modeling Covariates:** Covariates can be modeled as fixed or random and more accurately represent their true contribution in the model.

## Mixed Models - General

Mixed models are particularly useful in medical studies where a wide variety of factors influence the response to a treatment of interest. For example, suppose that an experimental treatment is being administered to a group of patients desiring to lose weight. Traditional statistical methodologies (e.g., ANOVA, multiple regression, etc.) require that the treatments be given at the same time intervals for all patients in the group in order for the statistical analysis and conclusions to be accurate. What would happen if patients were not all able to receive the treatment at the same time intervals or if some patients missed some treatments? Traditional statistical approaches would no longer be valid since there are random events or components entering into the experiment. This is where mixed models techniques become useful. A mixed model would allow us to make inferences about the treatment by modeling and estimating the random components. Furthermore, mixed models allow us to make greater use of incomplete data, such as that obtained from patients who drop out or miss scheduled treatments. Traditional methods would exclude such individuals from the analysis, losing valuable information.

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## What is a Mixed Model?

In a general linear model (GLM), a random sample of the individuals in each population is drawn. A treatment is applied to each individual in the sample and an outcome is measured. The data so obtained are analyzed using an analysis of variance table that produces an F-test.

A mathematical model may be formulated that underlies each analysis of variance. This model expresses the response variable as the sum of parameters of the population. For example, a linear model for a two-factor experiment could be

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$$

where  $i = 1, 2, \dots, I$  (the number of levels of factor 1),  $j = 1, 2, \dots, J$  (the number of levels of factor 2), and  $k = 1, 2, \dots, K$  (the number of subjects in the study). This model expresses the value of the response variable,  $Y$ , as the sum of five components:

- $\mu$       the mean.
- $a_i$       the contribution of the  $i^{\text{th}}$  level of a factor A.
- $b_j$       the contribution of the  $j^{\text{th}}$  level of a factor B.
- $(ab)_{ij}$    the combined contribution (or interaction) of the  $i^{\text{th}}$  level of a factor A and the  $j^{\text{th}}$  level of a factor B.
- $e_{ijk}$     the contribution of the  $k^{\text{th}}$  individual. This is often called the "error."

In this example, the linear model is made up of *fixed effects* only. An effect is fixed if the levels in the study represent all levels of the factor that are of interest, or at least all levels that are important for inference (e.g., treatment, dose, etc.).

The following assumptions are made when using the F-test in a general linear model.

1. The response variable is continuous.
2. The individuals are independent.
3. The  $e_{ijk}$  follow the normal probability distribution with mean equal to zero.
4. The variances of the  $e_{ijk}$  are equal for all values of  $i, j$ , and  $k$ .

## Mixed Models - General

The *Linear Mixed Model* (or just *Mixed Model*) is a natural extension of the general linear model. Mixed models extend linear models by allowing for the addition of *random effects*, where the levels of the factor represent a random subset of a larger group of all possible levels (e.g., time of administration, clinic, etc.). For example, the two-factor linear model above could be augmented to include a random block effect such as clinic or doctor since the clinic or doctor may be assumed to be a random realization from a distribution of clinics or doctors. Covariates (continuous) and/or nested effects can also be included in the mixed model to improve the accuracy of the fixed effect estimates. The general form of the mixed model in matrix notation is

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \boldsymbol{\varepsilon}$$

where

- y** vector of responses
- X** known design matrix of the fixed effects
- $\boldsymbol{\beta}$**  unknown vector of fixed effects parameters to be estimated
- Z** known design matrix of the random effects
- u** unknown vector of random effects
- $\boldsymbol{\varepsilon}$**  unobserved vector of random errors

We assume

$$\begin{aligned}\mathbf{u} &\sim N(\mathbf{0}, \mathbf{G}) \\ \boldsymbol{\varepsilon} &\sim N(\mathbf{0}, \mathbf{R}) \\ \text{Cov}[\mathbf{u}, \boldsymbol{\varepsilon}] &= \mathbf{0}\end{aligned}$$

where

- G** variance-covariance matrix of **u**
- R** variance-covariance matrix of the errors  **$\boldsymbol{\varepsilon}$**

The variance of **y**, denoted **V**, is

$$\begin{aligned}\mathbf{V} &= \text{Var}[\mathbf{y}] \\ &= \text{Var}[\mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \boldsymbol{\varepsilon}] \\ &= \mathbf{0} + \text{Var}[\mathbf{Z}\mathbf{u} + \boldsymbol{\varepsilon}] \\ &= \mathbf{Z}\mathbf{G}\mathbf{Z}' + \mathbf{R}\end{aligned}$$

In order to test the parameters in  **$\boldsymbol{\beta}$** , which is typically the goal in mixed model analysis, the unknown parameters ( **$\boldsymbol{\beta}$** , **G**, and **R**) must be estimated. Estimates for  **$\boldsymbol{\beta}$**  require estimates of **G** and **R**. In order to estimate **G** and **R**, the structure of **G** and **R** must be specified. Details of the specific structures for **G** and **R** are discussed later.

The following assumptions are made when using the F-test in a mixed model.

1. The response variable is continuous.
2. The individuals are independent.
3. The random error follows the normal probability distribution with mean equal to zero.

A distinct (and arguably the most important) advantage of the mixed model over the general linear model is flexibility in random error and random effect variance component modeling (note that the equal-variance assumption of the general linear model is not necessary for the linear mixed model). Mixed models allow you to model both heterogeneous variances and correlation among observations through the specification of the covariance matrix structures for  $\mathbf{u}$  and  $\boldsymbol{\epsilon}$ . You should be careful to build an appropriate covariance structure for the model, since the hypothesis tests, confidence intervals, and treatment mean estimates are all affected by the covariance structure of the model. The variance matrix estimates are obtained using maximum likelihood (ML) or, more commonly, restricted maximum likelihood (REML). The fixed effects in the mixed model are tested using F-tests. More details of random factor estimation and fixed factor estimation and testing are given later in this chapter.

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## Types of Mixed Models

Several general mixed model subtypes exist that are characterized by the random effects, fixed effects, covariate terms, and covariance structure they involve. These include fixed effects models, random effects models, covariance pattern models, and random coefficients models.

### Fixed Effects Models

A *fixed effects model* is a model where only fixed effects are included in the model. An effect (or factor) is fixed if the levels in the study represent all levels of interest of the factor, or at least all levels that are important for inference (e.g., treatment, dose, etc.). No random components are present. The general linear model is a fixed effects model. Fixed effects models can include covariates and/or interactions. The two-factor experiment example above gives an example of a fixed effects model. The fixed effects can be estimated and tested using the F-test. Fixed effects are specified as the Fixed Factors Model on the Variables tab.

Note: If only one response is recorded for each subject, there is no within-subject correlation to be modeled in variance-covariance matrix. If more than one response is measured for each subject, you could use repeated measures ANOVA or use a random-coefficients mixed model.

### Random Effects Models

A *random effects model* is a model with only random terms in the model. An effect (or factor) is random if the levels of the factor represent a random subset of a larger group of all possible levels (e.g., patients represent the population as a whole). Random effects are specified in the Subject (Random) Model box on the Variables tab. The random effects are not tested, but estimates are given.

Note: If only one response is recorded for each subject, there is no within-subject correlation to be modeled in variance-covariance matrix. If more than one response is measured for each subject, you could use repeated measures ANOVA or use a random coefficients mixed model.

## Longitudinal Data Models

Longitudinal data arises when more than one response is measured on each subject in the study. Responses are often measured over time at fixed or random intervals. An interval is fixed if the measurements are made at pre-specified time intervals, e.g., measuring heart rate after 2 hours, 4 hours, and 6 hours after drug administration. An interval is random if the response measurements are made at random time points, e.g., measuring heart rate at the start of a race and after each runner finishes (presumably at differing time points). Various covariance structures can be employed to model the variance and correlation among repeated measurements or the relationship with time can be investigated. The manner in which the longitudinal data is modeled gives rise to two different mixed model subtypes: covariance pattern models and random coefficients models.

### Covariance Pattern Models

If the covariance and correlation between repeated measurements is taken into account (i.e. modeled), the model is called a *covariance pattern model*. The covariance pattern model is usually appropriate if the repeated measurements occur at fixed intervals and the relationship with time is not of particular interest. More information is given later in the chapter about the different covariance patterns that can be fit.

The repeated or residual covariance pattern is specified in the Repeated Variance Pattern box on the Variables tab.

### Random Coefficients Models

It is often important in a study to determine the relationship between the response and time. This is often done by including the measurement time as a covariate in the model, with a corresponding slope, say  $\beta_t$ . It is plausible and likely that the slope will vary with subject, so it might be useful to model a separate intercept and slope for each subject in the study. This is done by fitting the subject variable as the intercept and the subject\*time interaction as the slope for each patient. These two terms could reasonably be assumed to arise at random from a distribution and, thus, would be specified as random effects. This gives rise to what is called a *random coefficients model*.

A random coefficients model is one in which the subject term and a subject\*time interaction term are both included as random effects in the model. This type of model is different from an ordinary random effects model because when we fit a straight line, the estimates of the slope and intercept are not independent. Thus, the subject and subject\*time effects in the model are correlated. The random effects model must be adapted to this situation to allow for correlation among these random effects. This is done using the bivariate normal distribution. The bivariate random effect becomes

$$\begin{pmatrix} \text{subject}_k \\ (\text{subject} * \text{time})_k \end{pmatrix} \sim N(0, \mathbf{G}),$$

where

$$\mathbf{G} = \begin{pmatrix} \sigma_{\text{subject}}^2 & \sigma_{\text{subject}, \text{subject} * \text{time}} \\ \sigma_{\text{subject}, \text{subject} * \text{time}} & \sigma_{\text{subject} * \text{time}}^2 \end{pmatrix}$$

The random coefficients model is usually used if the relationship with time is of interest or if the repeated measurements do not occur at fixed intervals. Random coefficient effects are specified in the Random Factor box on the Variables tab. Other fixed and random effects, besides time, can also be specified in the random coefficients model.

## Examples

Because of the large number of options, attempting to enter the appropriate model in the Mixed Models procedure can be intimidating. A number of examples at the end of the chapter are provided with the hope that one of the examples is similar enough to your scenario that it will guide you in selecting the options that are appropriate. The examples can also serve as a tutorial, beginning with the simplest two-group modeling in Example 2 (Example 1 is used for annotation) and continuing into more complex models.

Several of the examples also provide comparisons to analyses using classical procedures. For example, Example 3 compares the classical one-way analysis using the One-Way ANOVA procedure to the equivalent analysis using the Mixed Models procedure.

The examples at the end of this chapter are categorized in two ways.

1. The number of between-subject and within-subject factors
2. The experimental design or analysis method used

A brief explanation of between-subject factors and within-subject factors precedes the table of examples.

### Between-Subject Factors

Between-subject factors are those factors for which several subjects are assigned to (or sampled from) each level. If 12 subjects are randomly assigned to 3 treatment groups (4 subjects per group), treatment is a between-subject factor.

### Within-Subject Factors

Within-subject factors are those in which the subject's response is measured at several time points.

Within-subject factors are those factors for which multiple levels of the factor are measured on the same subject. If each subject is measured at the low, medium, and high level of the treatment, treatment is a within-subject factor.

### Example Overview

Example 1 has one within-subject factor and one between-subject factor, as well as a covariate. For Example 1, the output is annotated in detail. The remaining examples show the set-up and basic analysis.

Example	Design/Analysis	Number of Between-Subject Factors	Number of Within-Subject Factors	Number of Covariates
1	Repeated Measures (+ Annotation)	1	1	1
2a	Two-Group T-Test (Equal Variance)	1	0	0
2b	Two-Group T-Test (Unequal Variance)	1	0	0
2c	Two-Group T-Test (+ Covariate)	1	0	1
3a	One-Way (Equal Variance)	1	0	0
3b	One-Way (Unequal Variance)	1	0	0
4	One-Way (+ Covariate)	1	0	1
5	Factorial (+ Covariate)	2	0	1
6	RCBD	0	1	0
7	Complex Split-Plot	1	2	2
8	Cross-Over	0	2	1
9	Repeated Measures (Unequal Time Points)	1	0	1

## Random versus Repeated Error Formulation

The general form of the linear mixed model as described earlier is

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \boldsymbol{\varepsilon}$$

$$\mathbf{u} \sim N(\mathbf{0}, \mathbf{G})$$

$$\boldsymbol{\varepsilon} \sim N(\mathbf{0}, \mathbf{R})$$

$$\text{Cov}[\mathbf{u}, \boldsymbol{\varepsilon}] = \mathbf{0}$$

$$\mathbf{V} = \mathbf{Z}\mathbf{G}\mathbf{Z}' + \mathbf{R}$$

The specification of the random component of the model specifies the structure of  $\mathbf{Z}$ ,  $\mathbf{u}$ , and  $\mathbf{G}$ . The specification of the repeated (error or residual) component of the model specifies the structure of  $\boldsymbol{\varepsilon}$  and  $\mathbf{R}$ . Except in very complicated designs, it is recommended that only one of the two components be specified. That is, if the random component includes one or more terms, the repeated pattern should be the diagonal (basic) pattern. If the repeated pattern is more complicated than a diagonal, there should not be a random component. There are exceptions, but the resulting covariance structure should be carefully considered in such cases.

Specifying the random component of the model will suffice for most factorial, split-plot, and ANCOVA designs and for longitudinal designs with irregular time values. The repeated component of the model should be used for longitudinal analyses with a fixed number of time points (e.g., 1 hour, 2 hours, 4 hours, 8 hours), and where there are no, or very few, missing values.

In some scenarios, specifying a repeated pattern results in the same covariance parameter formulation as a random component. For example, specifying compound symmetry for the repeated pattern with no random component will result in the sample within-subject variance matrix as specifying Subject as the random factor and Diagonal for the repeated pattern. The examples of this chapter can be used to see the random and repeated specification for several common analyses.

## Determining the Correct Model of the Variance-Covariance of Y

### Akaike Information Criterion (AIC) for Model Assessment

Akaike information criterion (AIC) is tool for assessing model fit (Akaike, 1973, 1974). The formula is

$$AIC = -2 \times L + 2p$$

where  $L$  is the (ML or REML) log-likelihood and  $p$  depends on the type of likelihood selected. If the ML method is used,  $p$  is the total number of parameters. If the REML method is used,  $p$  is the number of variance component parameters.

The formula is designed so that a smaller AIC value indicates a "better" model. AIC penalizes models with larger numbers of parameters. That is, if a model with a much larger number of parameters produces only a slight improvement in likelihood, the values of AIC for the two models will suggest that the more parsimonious (limited) model is still the "better" model.

## Mixed Models - General

As an example, suppose a researcher would like to determine the appropriate variance-covariance structure for a longitudinal model with four equal time points. The researcher uses REML as the likelihood type. The analysis is run five times, each with a different covariance pattern, and the AIC values are recorded as follows.

Pattern	Number of Parameters	-2 log-likelihood	AIC
Diagonal	1	214.43	216.43
Compound Symmetry	2	210.77	214.77
AR(1)	2	203.52	207.52
Toeplitz	4	198.03	206.03
Unstructured	7	197.94	211.94

The recommended variance-covariance structure among these five is the Toeplitz pattern, since it results in the smallest AIC value.

### What to Do When You Encounter a Variance Estimate that is Equal to Zero

It is possible that a mixed models data analysis results in a variance component estimate that is negative or equal to zero. This is particularly true in the case of random coefficients models. When this happens, the component that has a variance estimate equal to zero should be removed from the random factors model statement (or, if possible, the repeated pattern should be simplified to 'diagonal'), and the analysis should be rerun.

As an example, suppose a researcher would like to analyze a dataset using a random coefficients model. The data consists of sixty subjects, each of which received one of three treatments. The weight of each subject was measured at the beginning of the study and 6, 12, 18, 24, and 30 days after administration of the treatment. The fixed and random factors models are entered as follows:

**Fixed Factors Model:** Day Trt Day\*Trt

**Random Factors Model:** Subject Subject\*Day

**Repeated (Time) Variance Pattern:** Diagonal

The mixed models analysis results in the following variance component parameter estimates:

#### Random Component Parameter Estimates (G Matrix)

Component Number	Parameter Number	Estimated Value	Model Term
1	1	<b>0.000000</b>	Subject
1	2	0.031682	Subject*Day



**Repeated Component Parameter Estimates (R Matrix)**

Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	12.914745	Diagonal (Variance)

\*\*\*\*\* RUN ABORTED BECAUSE OF ZERO PARAMETER \*\*\*\*\*

Error Explanation:

One or more of the above parameter estimates is zero.

The corresponding term should not be included in the model.

The term must be removed from the model and then the problem rerun in order to obtain the rest of the reports and charts.

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The estimated value for the Subject random component is equal to zero and should be removed from the analysis. Re-running the analysis without the Subject component in the random factors model results in the following parameter estimates:

**Random Component Parameter Estimates (G Matrix)**

Component Number	Parameter Number	Estimated Value	Model Term
1	1	0.030111	Subject*Day

**Repeated Component Parameter Estimates (R Matrix)**

Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	12.517215	Diagonal (Variance)

The variance estimates for the other parameters changed slightly after removing Subject from the random factors model.

## Fixed Effects

A fixed effect (or factor) is a variable for which levels in the study represent all levels of interest, or at least all levels that are important for inference (e.g., treatment, dose, etc.). The fixed effects in the model include those factor for which means, standard errors, and confidence intervals will be estimated and tests of hypotheses will be performed. Other variables for which the model is to be adjusted (that are not important for estimation or hypothesis testing) may also be included in the model as fixed factors. Fixed factors may be discrete variables or continuous covariates.

The correct model for fixed effects depends on the number of fixed factors, the questions to be answered by the analysis, and the amount of data available for the analysis. When more than one fixed factor may influence the response, it is common to include those factors in the model, along with their interactions (two-way, three-way, etc.). Difficulties arise when there are not sufficient data to model the higher-order interactions. In this case, some interactions must be omitted from the model. It is usually suggested that if you include an interaction in the model, you should also include the main effects (i.e., individual factors) involved in the interaction even if the hypothesis test for the main effects is not significant.

## Covariates

Covariates are continuous measurements that are not of primary interest in the study, but potentially have an influence on the response. Two types of covariates typically arise in mixed models designs: subject covariates and within-subject covariates. They are illustrated in the following example.

A study is conducted to determine the effect of two drugs on heart rate in mice. Each mouse receives each drug and a placebo with a washout period between treatments. The weight of each mouse is measured prior to the first treatment. The systolic blood pressure of each mouse is also measured immediately before each treatment. Although potentially an important factor, order of treatment is not considered in this example.

Mouse	IWeight	Treatment	BP	HR
1	18	Placebo	154	392
1	18	Drug A	167	378
1	18	Drug B	184	365
2	26	Placebo	166	402
2	26	Drug A	189	396
2	26	Drug B	177	397
3	22	Placebo	185	408
3	22	Drug A	163	402
3	22	Drug B	183	407
4	19	Placebo	167	411
4	19	Drug A	179	400
4	19	Drug B	172	392
5	15	Placebo	175	384
5	15	Drug A	168	391
5	15	Drug B	176	386
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.

In this example, initial weight (IWeight) and blood pressure (BP) are covariates. IWeight is a subject covariate because it is measured only once for each subject. BP is a within-subject covariate since it is measured on each subject for each treatment.

The Mixed Models procedure permits the user to make comparisons of fixed-effect means at specified values of covariates. For example, researchers could compare the two treatments to the placebo for IWeight = 20 and BP = 180, even when those values of the covariates do not appear in the actual data set.

Commonly, investigators wish to make comparisons of levels of a factor at several values of covariates. In this example, the researchers might want to compare the two treatments to the placebo at IWeight = 18, 23, and 26, and at BP = 160, 175, and 190. Caution should be exercised when making comparisons at multiple covariate values. The result in this case is  $3 \times 3 = 9$  sets of comparisons and, therefore,  $3 \times 9 = 27$  tests (3 pair wise treatment comparisons  $\times$  9 sets = 27 tests) for the Bonferroni adjustment of the p-value. After accounting for multiple testing, finding significant differences will require large sample sizes and/or extreme differences in means since the raw p-value would have to be less than  $0.00185$  in order to declare significance at the 0.05 level ( $0.05/27 = 0.00185$ ).

## Time as a Fixed Effects Factor vs. Time as a Covariate

Time is an essential measurement in many mixed model designs. In some analyses, time may be considered a fixed factor, while in others it is covariate. A couple of examples illustrate this distinction.

### Time as a Fixed Effects Factor

Researchers wish to compare the extent to which rashes develop following administration of different doses of an anti-fungal cream. Fifteen individuals are divided into three groups, with each group receiving a different dose of the cream: low, medium, or high. The surface area of the resulting rash is measured at four time points: 1 hour, 2 hours, 4 hours, and 8 hours.

Dose	Subject	Time	Rash
Low	1	1	4.2
Low	1	2	3.5
Low	1	4	2.1
Low	1	8	6.8
Low	2	1	3.4
Low	2	2	5.2
Low	2	4	9.7
Low	2	8	6.5
Low	3	1	4.1
Low	3	2	6.8
Low	3	4	7.1
Low	3	8	2.3
.	.	.	.
.	.	.	.
.	.	.	.
High	15	1	6.4
High	15	2	8.2
High	15	4	9.4
High	15	8	8.5

In this example, the time points are very structured (every subject is measured at the same time points) and the relationship between the size of the rash and time is not likely to be linear (the relationship will likely increase and then decrease). These two aspects of the study would generally lead the researcher to include Time as a fixed effects factor rather than as a covariate. If, however, the relationship were linear (or could be made linear by a suitable transformation), time could be considered a covariate. The next example examines the case where Time must be considered a covariate.

## Time as a Covariate

Three diets are compared for recently hatched chicks for their effect on growth. One hundred forty-seven chicks are randomly divided into three diets: low soybean protein, high soybean protein, and high fishmeal protein. Weights of chicks are measured at unequal times for two months after beginning the diet.

Diet	Chick	Time	Weight
Low Soy	1	5	64
Low Soy	1	11	69
Low Soy	1	24	74
Low Soy	1	45	101
Low Soy	2	16	72
Low Soy	2	51	143
Low Soy	3	3	57
Low Soy	3	29	81
Low Soy	3	33	83
Low Soy	3	46	126
Low Soy	3	55	155
Low Soy	4	8	72
.	.	.	.
.	.	.	.
.	.	.	.
High Fish	146	52	145
High Fish	147	16	78
High Fish	147	33	97
High Fish	146	52	145

In this example, if Time were considered a fixed-effects factor, each time point would be a different level of the factor, yielding too many levels. The appropriate approach in this example is to include Time as a covariate and examine the linear relationship (perhaps following a transformation) between Time and Weight. In this example, the nature of the design requires that Time be a covariate.

Common experiments in which time should be included as a covariate are experiments involving human subjects that don't report on schedule.

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## Using a Time Variable When Time is Not Measured in the Study

Many designs (e.g., factorial, split-plot, ANCOVA) for which the use of mixed models is recommended do not have time as a measured variable. In such cases, it can still be useful to include a time variable as an ordering variable. This is particularly important when the dataset itself is not ordered, when there are missing values, and when the specified covariance structure is complex. An example of a design where time is included only for ordering purposes is a cross-over design.

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## A Model-Building Strategy

There are three main components of a mixed model:

- **The Fixed Effects Component.** The fixed effects component of the model consists of the fixed factors, the covariates, and the interactions of fixed factors and covariates. The strength of evidence for the true effect of each fixed effects term is given by the probability level of the corresponding F-test.
- **The Random (Subject) Component.** The random factors include all random factors and (possibly) interactions of random factors with fixed factor variables or covariates. The importance of each random term is more subjective. Inclusion or exclusion of a random term is often decided by comparing the magnitude of the estimates. Relatively small estimates may, in some cases, be removed from the model. The meaning of 'relatively small' is beyond the scope of this manual.
- **The Covariance Pattern of Repeated Measurements.** The covariance pattern indicates the pattern of the residual error of repeated measurements. Specific patterns are shown in detail later in this chapter. The pattern should usually be Diagonal if a random model is specified. Patterns can be compared by examining the AIC value for each pattern. A separate run is required for each pattern.

The underlying goal in building a mixed model should be finding the simplest model that best fits the observed data. A reasonable top-down strategy for building a model might include the following steps:

1. Specify all the fixed effects, covariates, and potentially important interactions in the Fixed Effects Model.
2. Specify either the Random Model or the Repeated Covariance Pattern as the circumstances dictate.
3. Run the model.
4. Compare the random terms to see if any are clearly negligible (e.g., less than 20 times smaller than the others).
5. Re-run the model excluding the negligible random terms.
6. Examine the fixed effects terms F-tests tests. Iteratively remove interaction terms from the fixed effects model that have large probability levels until all are below, say, 0.20.
7. If a Repeated Covariance Pattern is of interest, re-run the analysis several times with different patterns, comparing the AIC values. Keep the pattern with the lowest AIC value.
8. Run the final model with comparisons of interest and specific covariate values.

This strategy is one among many that could be used in refining a mixed model. In some cases, regulations may dictate the terms that may or may not be included in the model, which leaves little or no room for refinement. The order of steps given here is subjective, but perhaps gives a feel for the considerations that should be made in determining a good model. The discussion near the end of Example 1 involving model refinement for a specific example may also be helpful.

## Multiple Comparisons of Fixed Effect Levels

If there is evidence that a fixed factor of a mixed model has difference responses among its levels, it is usually of interest to perform post-hoc pair-wise comparisons of the least-squares means to further clarify those differences. It is well-known that p-value adjustments need to be made when multiple tests are performed (see Hochberg and Tamhane, 1987, or Hsu, 1996, for general discussion and details of the need for multiplicity adjustment). Such adjustments are usually made to preserve the family-wise error rate (FWER), also called the experiment-wise error rate, of the group of tests. FWER is the probability of incorrectly rejecting at least one of the pair-wise tests.

### Family-Wise Error Rate (FWER) Control – Bonferroni Adjustment

The Bonferroni p-value adjustment produces adjusted p-values (probability levels) for which the FWER is controlled strictly (Westfall et al, 1999). The Bonferroni adjustment is applied to all  $m$  unadjusted (raw) p-values ( $p_j$ ) as

$$\tilde{p}_j = \min(mp_j, 1).$$

That is, each p-value is multiplied by the number of tests in the set (family), and if the result is greater than one, it is set to the maximum possible p-value of one.

The Bonferroni adjustment is generally considered to be a conservative method for simultaneously comparing levels of fixed effects.

In the following example, four levels of a fixed factor are compared (all pairs): A, B, C, and D.

#### Multiple Comparison Example – Main Effects

Test	Raw P-value	Bonferroni-Adjusted P-value
A vs B	0.01435	0.08610
A vs C	0.00762	0.04572
A vs D	0.00487	0.02922
B vs C	0.34981	1.00000
B vs D	0.06062	0.36372
C vs D	0.71405	1.00000

In this example, the adjustments are based on  $m = 6$  tests.

## Multiple Comparisons for the Interaction of Two Main Effects

When examining a fixed effect interaction using post-hoc (or planned) multiple comparison tests, a useful method is to compare all levels of one factor at each level of the other factor. This method is termed 'slicing'. For example, if the interaction of Time and Treatment is significant, comparing the treatment levels at each time point could aid in understanding the nature of the interaction.

### Multiple Comparison Example - Interaction

Time	Test	Raw P-value	Bonferroni-Adjusted P-value
1 hour	A vs B	0.25186	1.00000
1 hour	A vs C	0.00118	0.02124
1 hour	A vs D	0.13526	1.00000
1 hour	B vs C	0.07275	1.00000
1 hour	B vs D	0.12994	1.00000
1 hour	C vs D	0.08068	1.00000
5 hours	A vs B	0.11279	1.00000
5 hours	A vs C	0.01779	0.32022
5 hours	A vs D	0.18634	1.00000
5 hours	B vs C	0.07291	1.00000
5 hours	B vs D	0.05254	0.94572
5 hours	C vs D	0.03883	0.69894
10 hours	A vs B	0.14701	1.00000
10 hours	A vs C	0.02798	0.50364
10 hours	A vs D	0.15722	1.00000
10 hours	B vs C	0.13614	1.00000
10 hours	B vs D	0.10642	1.00000
10 hours	C vs D	0.16751	1.00000

In this example, the adjustments are based on  $m = 18$  tests. It can be seen from this example that minimizing the number of tests enhances the power to detect significant differences.

## Multiple Comparisons for Several Covariate Levels

When more than one covariate value is specified for 'Compute Means at these Values' on the Covariates tab, the number of test used in the Bonferroni adjustment can increase dramatically. The number of tests for the Bonferroni adjustment is computed as

$$\text{Number of Tests} = \text{Number of Comparisons per Set} \times \text{Number of Covariate Sets}$$

As an example, suppose that an experiment has two covariates, and a single fixed treatment factor with three levels: Control, T1, and T2. If 'All Pairs' were selected as the comparison on the Comparisons tab, then the number of comparisons per set would be three (T1 - Control, T2 - Control, and T2 - T1). Suppose that the researcher desired to compute the hypothesis tests at two values for the first covariate and four values for the second. The number of covariate sets would be  $2 \times 4 = 8$ . Therefore, the number of tests used in the Bonferroni adjustment to conserve the overall error-rate would be  $3 \times 8 = 24$ . The raw p-value would have to be less than  $0.05/24 = 0.00208$  in order to declare significance at the 0.05 level.

This example illustrates that care must be taken when specifying the covariate values at which the means and analyses will be computed. As more covariate values are specified, the number of tests in the adjustment increases making it more and more difficult to find differences that are significant.

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## Mixed Model Technical Details

As stated previously, the general form of the linear mixed model is

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \boldsymbol{\varepsilon}$$

where

- y** vector of responses
- X** known design matrix of the fixed effects
- $\boldsymbol{\beta}$**  unknown vector of fixed effects parameters to be estimated
- Z** known design matrix of the random effects
- u** unknown vector of random effects
- $\boldsymbol{\varepsilon}$**  unobserved vector of random errors

We assume

$$\begin{aligned}\mathbf{u} &\sim N(\mathbf{0}, \mathbf{G}) \\ \boldsymbol{\varepsilon} &\sim N(\mathbf{0}, \mathbf{R}) \\ \text{Cov}[\mathbf{u}, \boldsymbol{\varepsilon}] &= \mathbf{0}\end{aligned}$$

where

- G** variance-covariance matrix of **u**
- R** variance-covariance matrix of the errors  **$\boldsymbol{\varepsilon}$**

The variance of **y**, denoted **V**, is

$$\begin{aligned}\mathbf{V} &= \text{Var}[\mathbf{y}] \\ &= \text{Var}[\mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \boldsymbol{\varepsilon}] \\ &= \mathbf{0} + \text{Var}[\mathbf{Z}\mathbf{u} + \boldsymbol{\varepsilon}] \\ &= \mathbf{ZGZ}' + \mathbf{R}\end{aligned}$$

In order to test the parameters in  **$\boldsymbol{\beta}$** , which is typically the goal in mixed model analysis, the unknown parameters ( **$\boldsymbol{\beta}$** , **G**, and **R**) must be estimated. Estimates for  **$\boldsymbol{\beta}$**  require estimates of **G** and **R**. In order to estimate **G** and **R**, the structure of **G** and **R** must be specified. Structures for **G** and **R** are discussed later.



## Individual Subject Formulation

Because of the size of the matrices that are involved in mixed model analysis, it is useful for computational purposes to reduce the dimensionality of the problem by analyzing the data one subject at a time. Because the data from different subjects are statistically independent, the log-likelihood of the data can be summed over the subjects, according to the formulas below. Before we look at the likelihood functions, we examine the linear mixed model for a particular subject:

$$\mathbf{y}_i = \mathbf{X}_i\boldsymbol{\beta} + \mathbf{Z}_i\mathbf{u}_i + \boldsymbol{\varepsilon}_i, \quad i = 1, \dots, N$$

where

$\mathbf{y}_i$   $n_i \times 1$  vector of responses for subject  $i$ .

$\mathbf{X}_i$   $n_i \times p$  design matrix of fixed effects for subject  $i$  ( $p$  is the number of columns in  $\mathbf{X}$ ).

$\boldsymbol{\beta}$   $p \times 1$  vector of regression parameters.

$\mathbf{Z}_i$   $n_i \times q$  design matrix of the random effects for subject  $i$ .

$\mathbf{u}_i$   $q \times 1$  vector of random effects for subject  $i$  which has means of zero and covariance matrix  $\mathbf{G}_{sub}$ .

$\boldsymbol{\varepsilon}_i$   $n_i \times 1$  vector of errors for subject  $i$  with zero mean and covariance  $\mathbf{R}_i$ .

$n_i$  number of repeated measurements on subject  $i$ .

$N$  number of subjects.

The following definitions will also be useful.

$\mathbf{e}_i$  vector of residuals for subject  $i$  ( $\mathbf{e}_i = \mathbf{y}_i - \mathbf{X}_i\boldsymbol{\beta}$ )

$\mathbf{V}_i$   $\text{Var}[\mathbf{y}_i] = \mathbf{Z}_i\mathbf{G}_{sub}\mathbf{Z}_i' + \mathbf{R}_i$

To see how the individual subject mixed model formulation relates to the general form, we have

$$\mathbf{y} = \begin{pmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \\ \vdots \\ \mathbf{y}_N \end{pmatrix}, \quad \mathbf{X} = \begin{pmatrix} \mathbf{X}_1 \\ \mathbf{X}_2 \\ \vdots \\ \mathbf{X}_N \end{pmatrix}, \quad \mathbf{Z} = \begin{pmatrix} \mathbf{Z}_1 & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \ddots & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{Z}_N \end{pmatrix}, \quad \mathbf{u} = \begin{pmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \\ \vdots \\ \mathbf{u}_N \end{pmatrix}, \quad \boldsymbol{\varepsilon} = \begin{pmatrix} \boldsymbol{\varepsilon}_1 \\ \boldsymbol{\varepsilon}_2 \\ \vdots \\ \boldsymbol{\varepsilon}_N \end{pmatrix}$$

## Likelihood Formulas

Rather than maximizing the likelihood function, it is convenient (for theoretical and practical reasons beyond the scope of this manual) to minimize -2 times the log likelihood function rather than maximize the likelihood function itself. There are two types of likelihood estimation methods that are generally considered in mixed model estimation: maximum likelihood (ML) and restricted maximum likelihood (REML). REML is generally favored over ML because the variance estimates using REML are unbiased for small sample sizes, whereas ML estimates are unbiased only asymptotically (see Littell et al., 2006 or Demidenko, 2004). Both estimation methods are available in **NCSS**.

## Maximum Likelihood

The general form -2log-likelihood ML function is

$$-2L_{ML}(\boldsymbol{\beta}, \mathbf{G}, \mathbf{R}) = \ln|\mathbf{V}| + \mathbf{e}'\mathbf{V}^{-1}\mathbf{e} + N_T \ln(2\pi)$$

The equivalent individual subject form is

$$-2L_{ML}(\boldsymbol{\beta}, \mathbf{G}, \mathbf{R}) = \sum_{i=1}^N (\ln|\mathbf{V}_i| + \mathbf{e}'_i \mathbf{V}_i^{-1} \mathbf{e}_i) + N_T \ln(2\pi)$$

where  $N_T$  is the total number of observations, or

$$N_T = \sum_{i=1}^N n_i$$

## Restricted Maximum Likelihood

The general form -2log-likelihood REML function is

$$-2L_{REML}(\boldsymbol{\beta}, \mathbf{G}, \mathbf{R}) = \ln|\mathbf{V}| + \mathbf{e}'\mathbf{V}^{-1}\mathbf{e} + \ln|\mathbf{X}'\mathbf{V}^{-1}\mathbf{X}| + (N_T - p) \ln(2\pi)$$

The equivalent individual subject form is

$$-2L_{REML}(\boldsymbol{\beta}, \mathbf{G}, \mathbf{R}) = \sum_{i=1}^N [\ln|\mathbf{V}_i| + \mathbf{e}'_i \mathbf{V}_i^{-1} \mathbf{e}_i] + \ln \left| \sum_{i=1}^N \mathbf{X}'_i \mathbf{V}_i^{-1} \mathbf{X}_i \right| + (N_T - p) \ln(2\pi)$$

where, again,  $N_T$  is the total number of observations, or

$$N_T = \sum_{i=1}^N n_i$$

and  $p$  is the number of columns in  $\mathbf{X}$  or  $\mathbf{X}_i$ .

## The G Matrix

The  $\mathbf{G}$  matrix is the variance-covariance matrix for the random effects  $\mathbf{u}$ . Typically, when the  $\mathbf{G}$  matrix is used to specify the variance-covariance structure of  $\mathbf{y}$ , the structure for  $\mathbf{R}$  is simply  $\sigma^2\mathbf{I}$ . Caution should be used when both  $\mathbf{G}$  and  $\mathbf{R}$  are specified as complex structures, since large numbers of sometimes redundant covariance elements can result.

The  $\mathbf{G}$  matrix is made up of  $N$  symmetric  $\mathbf{G}_{sub}$  matrices,

$$\mathbf{G} = \begin{pmatrix} \mathbf{G}_{sub} & \mathbf{0} & \mathbf{0} & \cdots & \mathbf{0} \\ \mathbf{0} & \mathbf{G}_{sub} & \mathbf{0} & \cdots & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{G}_{sub} & \cdots & \mathbf{0} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \cdots & \mathbf{G}_{sub} \end{pmatrix}$$

## Mixed Models - General

The dimension of  $\mathbf{G}_{sub}$  is  $q \times q$ , where  $q$  is the number of random effects for each subject.

Structures of  $\mathbf{G}_{sub}$ 

There are two commonly used structures for the elements of the  $\mathbf{G}_{sub}$  matrix: diagonal and unstructured.

$$\begin{array}{cc} \text{Diagonal } \mathbf{G}_{sub} & \text{Unstructured } \mathbf{G}_{sub} \\ \mathbf{G}_{sub} = \begin{pmatrix} \sigma_1^2 & & & \\ & \sigma_2^2 & & \\ & & \sigma_3^2 & \\ & & & \sigma_4^2 \end{pmatrix} & \mathbf{G}_{sub} = \begin{pmatrix} \sigma_1^2 & \sigma_{12} & \sigma_{13} & \sigma_{14} \\ \sigma_{21} & \sigma_2^2 & \sigma_{23} & \sigma_{24} \\ \sigma_{31} & \sigma_{32} & \sigma_3^2 & \sigma_{34} \\ \sigma_{41} & \sigma_{42} & \sigma_{43} & \sigma_4^2 \end{pmatrix} \end{array}$$

The diagonal  $\mathbf{G}_{sub}$  should be used when there is no covariance between parameters, such as in the random effects models. The unstructured  $\mathbf{G}_{sub}$  is typically used when you want to include covariances, such as in random coefficients models.

The  $\mathbf{R}$  Matrix

The  $\mathbf{R}$  matrix is the variance-covariance matrix for errors,  $\boldsymbol{\varepsilon}$ . When the  $\mathbf{R}$  matrix is used to specify the variance-covariance structure of  $\mathbf{y}$ , the  $\mathbf{G}_{sub}$  matrix is not used.

The full  $\mathbf{R}$  matrix is made up of  $N$  symmetric  $\mathbf{R}$  sub-matrices,

$$\mathbf{R} = \begin{pmatrix} \mathbf{R}_1 & \mathbf{0} & \mathbf{0} & \cdots & \mathbf{0} \\ \mathbf{0} & \mathbf{R}_2 & \mathbf{0} & \cdots & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{R}_3 & \cdots & \mathbf{0} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \cdots & \mathbf{R}_N \end{pmatrix},$$

where  $\mathbf{R}_1, \mathbf{R}_2, \mathbf{R}_3, \dots, \mathbf{R}_N$  are all of the same structure, but, unlike the  $\mathbf{G}_{sub}$  matrices, differ according to the number of repeated measurements on each subject.

When the  $\mathbf{R}$  matrix is specified in **NCSS**, it is assumed that there is a fixed, known set of repeated measurement times. (If the repeated measurement times are random, specification of the  $\mathbf{G}_{sub}$  matrix with  $\mathbf{R} = \sigma^2 \mathbf{I}$  should be used instead for specifying covariance structure.) Thus, the differences in the dimensions of the  $\mathbf{R}$  sub-matrices occur only when some measurements for a subject are missing.

As an example, suppose an  $\mathbf{R}$  sub-matrix is of the form

$$\mathbf{R}_{sub} = \begin{pmatrix} \sigma_1^2 & & & & \\ & \sigma_2^2 & & & \\ & & \sigma_3^2 & & \\ & & & \sigma_4^2 & \\ & & & & \sigma_5^2 \end{pmatrix},$$

where there are five time points at which each subject is intended to be measured: 1 hour, 2 hours, 5 hours, 10 hours, and 24 hours. If the first subject has measurements at all five time points, then  $n_1 = 5$ , and the sub-matrix is identical to  $\mathbf{R}_{sub}$  above, and  $\mathbf{R}_1 = \mathbf{R}_{sub}$ .

## Mixed Models - General

Suppose the second subject is measured at 1 hour, 5 hours, and 24 hours, but misses the 2-hour and 10-hour measurements. The  $\mathbf{R}_2$  matrix for this subject is

$$\mathbf{R}_2 = \begin{pmatrix} \sigma_1^2 & & \\ & \sigma_3^2 & \\ & & \sigma_5^2 \end{pmatrix}.$$

For this subject,  $n_2 = 3$ . That is, for the case when the time points are fixed, instead of having missing values in the  $\mathbf{R}$  sub-matrices, the matrix is collapsed to accommodate the number of realized measurements.

Structures of  $\mathbf{R}$ 

There are many possible structures for the sub-matrices that make up the  $\mathbf{R}$  matrix. The  $\mathbf{R}_{sub}$  structures that can be specified in **NCSS** are shown below.

*Diagonal*

Homogeneous

$$\begin{pmatrix} \sigma^2 & & & \\ & \sigma^2 & & \\ & & \sigma^2 & \\ & & & \sigma^2 \end{pmatrix}$$

Heterogeneous

$$\begin{pmatrix} \sigma_1^2 & & & \\ & \sigma_2^2 & & \\ & & \sigma_3^2 & \\ & & & \sigma_4^2 \end{pmatrix}$$

Correlation

$$\begin{pmatrix} 1 & & & \\ & 1 & & \\ & & 1 & \\ & & & 1 \end{pmatrix}$$

*Compound Symmetry*

Homogeneous

$$\begin{pmatrix} \sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 \\ \rho\sigma^2 & \sigma^2 & \rho\sigma^2 & \rho\sigma^2 \\ \rho\sigma^2 & \rho\sigma^2 & \sigma^2 & \rho\sigma^2 \\ \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \sigma^2 \end{pmatrix}$$

Heterogeneous

$$\begin{pmatrix} \sigma_1^2 & \rho\sigma_1\sigma_2 & \rho\sigma_1\sigma_3 & \rho\sigma_1\sigma_4 \\ \rho\sigma_2\sigma_1 & \sigma_2^2 & \rho\sigma_2\sigma_3 & \rho\sigma_2\sigma_4 \\ \rho\sigma_3\sigma_1 & \rho\sigma_3\sigma_2 & \sigma_3^2 & \rho\sigma_3\sigma_4 \\ \rho\sigma_4\sigma_1 & \rho\sigma_4\sigma_2 & \rho\sigma_4\sigma_3 & \sigma_4^2 \end{pmatrix}$$

Correlation

$$\begin{pmatrix} 1 & \rho & \rho & \rho \\ \rho & 1 & \rho & \rho \\ \rho & \rho & 1 & \rho \\ \rho & \rho & \rho & 1 \end{pmatrix}$$

*AR(1)*

Homogeneous

$$\begin{pmatrix} \sigma^2 & \rho\sigma^2 & \rho^2\sigma^2 & \rho^3\sigma^2 \\ \rho\sigma^2 & \sigma^2 & \rho\sigma^2 & \rho^2\sigma^2 \\ \rho^2\sigma^2 & \rho\sigma^2 & \sigma^2 & \rho\sigma^2 \\ \rho^3\sigma^2 & \rho^2\sigma^2 & \rho\sigma^2 & \sigma^2 \end{pmatrix}$$

Heterogeneous

$$\begin{pmatrix} \sigma_1^2 & \rho\sigma_1\sigma_2 & \rho^2\sigma_1\sigma_3 & \rho^3\sigma_1\sigma_4 \\ \rho\sigma_2\sigma_1 & \sigma_2^2 & \rho\sigma_2\sigma_3 & \rho^2\sigma_2\sigma_4 \\ \rho^2\sigma_3\sigma_1 & \rho\sigma_3\sigma_2 & \sigma_3^2 & \rho\sigma_3\sigma_4 \\ \rho^3\sigma_4\sigma_1 & \rho^2\sigma_4\sigma_2 & \rho\sigma_4\sigma_3 & \sigma_4^2 \end{pmatrix}$$

Correlation

$$\begin{pmatrix} 1 & \rho & \rho^2 & \rho^3 \\ \rho & 1 & \rho & \rho^2 \\ \rho^2 & \rho & 1 & \rho \\ \rho^3 & \rho^2 & \rho & 1 \end{pmatrix}$$

Mixed Models - General

AR(Time Diff)

Homogeneous

$$\begin{pmatrix} \sigma^2 & \rho^{t_2-t_1}\sigma^2 & \rho^{t_3-t_1}\sigma^2 & \rho^{t_4-t_1}\sigma^2 \\ \rho^{t_2-t_1}\sigma^2 & \sigma^2 & \rho^{t_3-t_2}\sigma^2 & \rho^{t_4-t_2}\sigma^2 \\ \rho^{t_3-t_1}\sigma^2 & \rho^{t_3-t_2}\sigma^2 & \sigma^2 & \rho^{t_4-t_3}\sigma^2 \\ \rho^{t_4-t_1}\sigma^2 & \rho^{t_4-t_2}\sigma^2 & \rho^{t_4-t_3}\sigma^2 & \sigma^2 \end{pmatrix}$$

Heterogeneous

$$\begin{pmatrix} \sigma_1^2 & \rho^{t_2-t_1}\sigma_1\sigma_2 & \rho^{t_3-t_1}\sigma_1\sigma_3 & \rho^{t_4-t_1}\sigma_1\sigma_4 \\ \rho^{t_2-t_1}\sigma_2\sigma_1 & \sigma_2^2 & \rho^{t_3-t_2}\sigma_2\sigma_3 & \rho^{t_4-t_2}\sigma_2\sigma_4 \\ \rho^{t_3-t_1}\sigma_3\sigma_1 & \rho^{t_3-t_2}\sigma_3\sigma_2 & \sigma_3^2 & \rho^{t_4-t_3}\sigma_3\sigma_4 \\ \rho^{t_4-t_1}\sigma_4\sigma_1 & \rho^{t_4-t_2}\sigma_4\sigma_2 & \rho^{t_4-t_3}\sigma_4\sigma_3 & \sigma_4^2 \end{pmatrix}$$

Correlation

$$\begin{pmatrix} 1 & \rho^{t_2-t_1} & \rho^{t_3-t_1} & \rho^{t_4-t_1} \\ \rho^{t_2-t_1} & 1 & \rho^{t_3-t_2} & \rho^{t_4-t_2} \\ \rho^{t_3-t_1} & \rho^{t_3-t_2} & 1 & \rho^{t_4-t_3} \\ \rho^{t_4-t_1} & \rho^{t_4-t_2} & \rho^{t_4-t_3} & 1 \end{pmatrix}$$

Toeplitz

Homogeneous

$$\begin{pmatrix} \sigma^2 & \rho_1\sigma^2 & \rho_2\sigma^2 & \rho_3\sigma^2 \\ \rho_1\sigma^2 & \sigma^2 & \rho_1\sigma^2 & \rho_2\sigma^2 \\ \rho_2\sigma^2 & \rho_1\sigma^2 & \sigma^2 & \rho_1\sigma^2 \\ \rho_3\sigma^2 & \rho_2\sigma^2 & \rho_1\sigma^2 & \sigma^2 \end{pmatrix}$$

Heterogeneous

$$\begin{pmatrix} \sigma_1^2 & \rho_1\sigma_1\sigma_2 & \rho_2\sigma_1\sigma_3 & \rho_3\sigma_1\sigma_4 \\ \rho_1\sigma_2\sigma_1 & \sigma_2^2 & \rho_1\sigma_2\sigma_3 & \rho_2\sigma_2\sigma_4 \\ \rho_2\sigma_3\sigma_1 & \rho_1\sigma_3\sigma_2 & \sigma_3^2 & \rho_1\sigma_3\sigma_4 \\ \rho_3\sigma_4\sigma_1 & \rho_2\sigma_4\sigma_2 & \rho_1\sigma_4\sigma_3 & \sigma_4^2 \end{pmatrix}$$

Correlation

$$\begin{pmatrix} 1 & \rho_1 & \rho_2 & \rho_3 \\ \rho_1 & 1 & \rho_1 & \rho_2 \\ \rho_2 & \rho_1 & 1 & \rho_1 \\ \rho_3 & \rho_2 & \rho_1 & 1 \end{pmatrix}$$

Toeplitz(2)

Homogeneous

$$\begin{pmatrix} \sigma^2 & \rho_1\sigma^2 & & & \\ \rho_1\sigma^2 & \sigma^2 & \rho_1\sigma^2 & & \\ & \rho_1\sigma^2 & \sigma^2 & \rho_1\sigma^2 & \\ & & \rho_1\sigma^2 & \sigma^2 & \end{pmatrix}$$

Heterogeneous

$$\begin{pmatrix} \sigma_1^2 & \rho_1\sigma_1\sigma_2 & & & \\ \rho_1\sigma_2\sigma_1 & \sigma_2^2 & \rho_1\sigma_2\sigma_3 & & \\ & \rho_1\sigma_3\sigma_2 & \sigma_3^2 & \rho_1\sigma_3\sigma_4 & \\ & & \rho_1\sigma_4\sigma_3 & \sigma_4^2 & \end{pmatrix}$$

Correlation

$$\begin{pmatrix} 1 & \rho_1 & & & \\ \rho_1 & 1 & \rho_1 & & \\ & \rho_1 & 1 & \rho_1 & \\ & & \rho_1 & 1 & \end{pmatrix}$$

Note: This is the same as Banded(2).

Toeplitz(3)

Homogeneous

$$\begin{pmatrix} \sigma^2 & \rho_1\sigma^2 & \rho_2\sigma^2 & & \\ \rho_1\sigma^2 & \sigma^2 & \rho_1\sigma^2 & \rho_2\sigma^2 & \\ \rho_2\sigma^2 & \rho_1\sigma^2 & \sigma^2 & \rho_1\sigma^2 & \\ & \rho_2\sigma^2 & \rho_1\sigma^2 & \sigma^2 & \end{pmatrix}$$

Heterogeneous

$$\begin{pmatrix} \sigma_1^2 & \rho_1\sigma_1\sigma_2 & \rho_2\sigma_1\sigma_3 & & \\ \rho_1\sigma_2\sigma_1 & \sigma_2^2 & \rho_1\sigma_2\sigma_3 & \rho_2\sigma_2\sigma_4 & \\ \rho_2\sigma_3\sigma_1 & \rho_1\sigma_3\sigma_2 & \sigma_3^2 & \rho_1\sigma_3\sigma_4 & \\ & \rho_2\sigma_4\sigma_2 & \rho_1\sigma_4\sigma_3 & \sigma_4^2 & \end{pmatrix}$$

Correlation

$$\begin{pmatrix} 1 & \rho_1 & \rho_2 & & \\ \rho_1 & 1 & \rho_1 & \rho_2 & \\ \rho_2 & \rho_1 & 1 & \rho_1 & \\ & \rho_2 & \rho_1 & 1 & \end{pmatrix}$$

Toeplitz(4) and Toeplitz(5)

Toeplitz(4) and Toeplitz(5) follow the same pattern as Toeplitz(2) and Toeplitz(3), but with the corresponding numbers of bands.

*Banded(2)*

Homogeneous	Heterogeneous	Correlation
$\begin{pmatrix} \sigma^2 & \rho_1\sigma^2 & & \\ \rho_1\sigma^2 & \sigma^2 & \rho_1\sigma^2 & \\ & \rho_1\sigma^2 & \sigma^2 & \rho_1\sigma^2 \\ & & \rho_1\sigma^2 & \sigma^2 \end{pmatrix}$	$\begin{pmatrix} \sigma_1^2 & \rho_1\sigma_1\sigma_2 & & \\ \rho_1\sigma_2\sigma_1 & \sigma_2^2 & \rho_1\sigma_2\sigma_3 & \\ & \rho_1\sigma_3\sigma_2 & \sigma_3^2 & \rho_1\sigma_3\sigma_4 \\ & & \rho_1\sigma_4\sigma_3 & \sigma_4^2 \end{pmatrix}$	$\begin{pmatrix} 1 & \rho_1 & & \\ \rho_1 & 1 & \rho_1 & \\ & \rho_1 & 1 & \rho_1 \\ & & \rho_1 & 1 \end{pmatrix}$

Note: This is the same as Toeplitz(2).

*Banded(3)*

Homogeneous	Heterogeneous	Correlation
$\begin{pmatrix} \sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \\ \rho\sigma^2 & \sigma^2 & \rho\sigma^2 & \rho\sigma^2 \\ \rho\sigma^2 & \rho\sigma^2 & \sigma^2 & \rho\sigma^2 \\ & \rho\sigma^2 & \rho\sigma^2 & \sigma^2 \end{pmatrix}$	$\begin{pmatrix} \sigma_1^2 & \rho\sigma_1\sigma_2 & \rho\sigma_1\sigma_3 & \\ \rho\sigma_2\sigma_1 & \sigma_2^2 & \rho\sigma_2\sigma_3 & \rho\sigma_2\sigma_4 \\ \rho\sigma_3\sigma_1 & \rho\sigma_3\sigma_2 & \sigma_3^2 & \rho\sigma_3\sigma_4 \\ & \rho\sigma_4\sigma_2 & \rho\sigma_4\sigma_3 & \sigma_4^2 \end{pmatrix}$	$\begin{pmatrix} 1 & \rho & \rho & \\ \rho & 1 & \rho & \rho \\ \rho & \rho & 1 & \rho \\ & \rho & \rho & 1 \end{pmatrix}$

*Banded(4) and Banded (5)*

Banded(4) and Banded(5) follow the same pattern as Banded(2) and Banded(3), but with the corresponding numbers of bands.

*Unstructured*

Homogeneous	Heterogeneous	Correlation
$\begin{pmatrix} \sigma^2 & \rho_{12}\sigma^2 & \rho_{13}\sigma^2 & \rho_{14}\sigma^2 \\ \rho_{21}\sigma^2 & \sigma^2 & \rho_{23}\sigma^2 & \rho_{24}\sigma^2 \\ \rho_{31}\sigma^2 & \rho_{32}\sigma^2 & \sigma^2 & \rho_{34}\sigma^2 \\ \rho_{41}\sigma^2 & \rho_{42}\sigma^2 & \rho_{43}\sigma^2 & \sigma^2 \end{pmatrix}$	$\begin{pmatrix} \sigma_1^2 & \rho_{12}\sigma_1\sigma_2 & \rho_{13}\sigma_1\sigma_3 & \rho_{14}\sigma_1\sigma_4 \\ \rho_{21}\sigma_2\sigma_1 & \sigma_2^2 & \rho_{23}\sigma_2\sigma_3 & \rho_{24}\sigma_2\sigma_4 \\ \rho_{31}\sigma_3\sigma_1 & \rho_{32}\sigma_3\sigma_2 & \sigma_3^2 & \rho_{34}\sigma_3\sigma_4 \\ \rho_{41}\sigma_4\sigma_1 & \rho_{42}\sigma_4\sigma_2 & \rho_{43}\sigma_4\sigma_3 & \sigma_4^2 \end{pmatrix}$	$\begin{pmatrix} 1 & \rho_{12} & \rho_{13} & \rho_{14} \\ \rho_{21} & 1 & \rho_{23} & \rho_{24} \\ \rho_{31} & \rho_{32} & 1 & \rho_{34} \\ \rho_{41} & \rho_{42} & \rho_{43} & 1 \end{pmatrix}$

**Partitioning the Variance-Covariance Structure with Groups**

In the case where it is expected that the variance-covariance parameters are different across group levels of the data, it may be useful to specify a different set of **R** or **G** parameters for each level of a group variable. This produces a set of variance-covariance parameters that is different for each level of the chosen group variable, but each set has the same structure as the other groups.

**Partitioning the G Matrix Parameters**

Suppose the structure of **G** is specified to be diagonal. If **G<sub>sub</sub>** has four parameters, then

$$\mathbf{G}_{sub} = \begin{pmatrix} \sigma_1^2 & & & \\ & \sigma_2^2 & & \\ & & \sigma_3^2 & \\ & & & \sigma_4^2 \end{pmatrix}.$$

## Mixed Models - General

If there are twenty subjects, then

$$\mathbf{G} = \begin{pmatrix} \mathbf{G}_{sub} & 0 & 0 & \cdots & 0 \\ 0 & \mathbf{G}_{sub} & 0 & \cdots & 0 \\ 0 & 0 & \mathbf{G}_{sub} & \cdots & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & 0 & \cdots & \mathbf{G}_{sub} \end{pmatrix}.$$

The total number of variance parameters is four.

Suppose now that there are two groups of ten subjects, and it is believed that the four variance parameters of the first group are different from the four variance parameters of the second group.

We now have

$$\mathbf{G}_1 = \begin{pmatrix} \sigma_{11}^2 & & & \\ & \sigma_{12}^2 & & \\ & & \sigma_{13}^2 & \\ & & & \sigma_{14}^2 \end{pmatrix}, \text{ and } \mathbf{G}_2 = \begin{pmatrix} \sigma_{21}^2 & & & \\ & \sigma_{22}^2 & & \\ & & \sigma_{23}^2 & \\ & & & \sigma_{24}^2 \end{pmatrix}.$$

If the first ten subjects are in Group 1, then the  $\mathbf{G}$  matrix becomes

$$\mathbf{G} = \begin{pmatrix} \mathbf{G}_1 & & & & & & & \\ & \mathbf{G}_1 & & & & & & \\ & & \ddots & & & & & \\ & & & \mathbf{G}_1 & & & & \\ & & & & \mathbf{G}_2 & & & \\ & & & & & \mathbf{G}_2 & & \\ & & & & & & \ddots & \\ & & & & & & & \mathbf{G}_2 \end{pmatrix},$$

with eight variance parameters, rather than four.

### Partitioning the R Matrix Parameters

Suppose the structure of  $\mathbf{R}$  in a study with four time points is specified to be Toeplitz:

$$\mathbf{R} = \begin{pmatrix} \sigma^2 & \rho_1\sigma^2 & \rho_2\sigma^2 & \rho_3\sigma^2 \\ \rho_1\sigma^2 & \sigma^2 & \rho_1\sigma^2 & \rho_2\sigma^2 \\ \rho_2\sigma^2 & \rho_1\sigma^2 & \sigma^2 & \rho_1\sigma^2 \\ \rho_3\sigma^2 & \rho_2\sigma^2 & \rho_1\sigma^2 & \sigma^2 \end{pmatrix}.$$

If there are sixteen subjects, then

$$\mathbf{R} = \begin{pmatrix} \mathbf{R}_1 & \mathbf{0} & \mathbf{0} & \cdots & \mathbf{0} \\ \mathbf{0} & \mathbf{R}_2 & \mathbf{0} & \cdots & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{R}_3 & \cdots & \mathbf{0} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \cdots & \mathbf{R}_{16} \end{pmatrix}.$$

## Mixed Models - General

The total number of variance-covariance parameters is four:  $\sigma^2$ ,  $\rho_1$ ,  $\rho_2$ , and  $\rho_3$ .

Suppose now that there are two groups of eight subjects, and it is believed that the four variance parameters of the first group are different from the four variance parameters of the second group.

We now have

$$\mathbf{R}_1, \dots, \mathbf{R}_8 = \begin{pmatrix} \sigma_1^2 & \rho_{11}\sigma^2 & \rho_{12}\sigma^2 & \rho_{13}\sigma^2 \\ \rho_{11}\sigma^2 & \sigma_1^2 & \rho_{11}\sigma^2 & \rho_{12}\sigma^2 \\ \rho_{12}\sigma^2 & \rho_{11}\sigma^2 & \sigma_1^2 & \rho_{11}\sigma^2 \\ \rho_{13}\sigma^2 & \rho_{12}\sigma^2 & \rho_{11}\sigma^2 & \sigma_1^2 \end{pmatrix},$$

and

$$\mathbf{R}_9, \dots, \mathbf{R}_{16} = \begin{pmatrix} \sigma_2^2 & \rho_{21}\sigma^2 & \rho_{22}\sigma^2 & \rho_{23}\sigma^2 \\ \rho_{21}\sigma^2 & \sigma_2^2 & \rho_{21}\sigma^2 & \rho_{22}\sigma^2 \\ \rho_{22}\sigma^2 & \rho_{21}\sigma^2 & \sigma_2^2 & \rho_{21}\sigma^2 \\ \rho_{23}\sigma^2 & \rho_{22}\sigma^2 & \rho_{21}\sigma^2 & \sigma_2^2 \end{pmatrix}.$$

The total number of variance-covariance parameters is now eight.

It is easy to see how quickly the number of variance-covariance parameters increases when  $\mathbf{R}$  or  $\mathbf{G}$  is partitioned by groups.

### Repeated Measures Complication in Partitioning $\mathbf{R}$

When partitioning the variance-covariance parameters into groups in some less-common repeated-measures designs, more than one group can occur within a subject. Re-examining the  $\mathbf{R}$  partitioning example above, suppose instead that all sixteen subjects are measured four times: twice with Treatment A, and twice with Treatment B. For the sake of this example, assume that the first eight subjects receive A, A, B, B and the second eight receive B, B, A, A. The covariance parameters across treatments but within a subject are assumed to be zero, and the  $\mathbf{R}$  sub-matrices for the first eight subjects become

$$\mathbf{R}_1, \dots, \mathbf{R}_8 = \begin{pmatrix} \sigma_A^2 & \rho_A\sigma_A^2 & & \\ \rho_A\sigma_A^2 & \sigma_A^2 & & \\ & & \sigma_B^2 & \rho_B\sigma_B^2 \\ & & \rho_B\sigma_B^2 & \sigma_B^2 \end{pmatrix},$$

and for the last eight subjects,

$$\mathbf{R}_9, \dots, \mathbf{R}_{16} = \begin{pmatrix} \sigma_B^2 & \rho_B\sigma_B^2 & & \\ \rho_B\sigma_B^2 & \sigma_B^2 & & \\ & & \sigma_A^2 & \rho_A\sigma_A^2 \\ & & \rho_A\sigma_A^2 & \sigma_A^2 \end{pmatrix}.$$

The total number of variance-covariance parameters is only four:  $\sigma_A^2$ ,  $\sigma_B^2$ ,  $\rho_A$ , and  $\rho_B$ .

In general, when we attempt to divide the variance-covariance parameters into groups with a repeated-measures design, the covariance of residuals within a subject, but across treatments, is assumed to be zero.



## Estimating and Testing Fixed Effects Parameters

The estimation phase in the analysis of a mixed model produces variance and covariance parameter estimates of the elements of  $\mathbf{G}$  and  $\mathbf{R}$ , giving  $\hat{\mathbf{R}}$  and  $\hat{\mathbf{G}}$ , and hence,  $\hat{\mathbf{V}}$ . The REML and ML solutions for  $\hat{\boldsymbol{\beta}}$  are given by

$$\hat{\boldsymbol{\beta}} = (\mathbf{X}'\hat{\mathbf{V}}^{-1}\mathbf{X})^{-1}\mathbf{X}'\hat{\mathbf{V}}^{-1}\mathbf{y}$$

with estimated variance-covariance

$$\hat{\boldsymbol{\Sigma}} = \text{var}(\hat{\boldsymbol{\beta}}) = (\mathbf{X}'\hat{\mathbf{V}}^{-1}\mathbf{X})^{-1}$$

See, for example, Brown and Prescott (2006), Muller and Stewart (2006), or Demidenko (2004) for more details of the estimating equations.

Hypothesis tests and confidence intervals for  $\boldsymbol{\beta}$  are formed using a linear combination matrix (or vector)  $\mathbf{L}$ .

### L Matrix Details

$\mathbf{L}$  matrices specify linear combinations of  $\boldsymbol{\beta}$  corresponding to means or hypothesis tests of interest. Essentially, the  $\mathbf{L}$  matrix defines the mean or test. The number of columns in each  $\mathbf{L}$  matrix is the same as the number of elements of  $\boldsymbol{\beta}$ . For estimating a particular mean, the  $\mathbf{L}$  matrix consists of a single row. For hypothesis tests, the number of rows of  $\mathbf{L}$  varies according to the test. Below are some examples of  $\mathbf{L}$  matrices that arise in common analyses:

#### L Matrix for Testing a Single Factor (Food with 4 levels) in a Single-Factor Model

No.	Effect	Food	L1	L2	L3
1	Intercept				
2	Food	HighIron	1.0000	1.0000	1.0000
3	Food	LowIron	-1.0000		
4	Food	None		-1.0000	
5	Food	Salicyl			-1.0000

#### L Matrix for a Single Mean (LowIron) of a Single Factor (4 levels) in a Single-Factor Model

No.	Effect	Food	L1
1	Intercept		1.0000
2	Food	HighIron	
3	Food	LowIron	1.0000
4	Food	None	
5	Food	Salicyl	

## Mixed Models - General

### L Matrix for Testing a Single Factor (Drug – 3 levels) in a Two-Factor Model with Interaction

No.	Effect	Drug	Time	L1	L2
1	Intercept				
2	Drug	Kerlosin		1.0000	1.0000
3	Drug	Laposec		-1.0000	
4	Drug	Placebo			-1.0000
5	Time		0.5		
6	Time		1		
7	Time		1.5		
8	Time		2		
9	Time		2.5		
10	Time		3		
11	Drug*Time	Kerlosin	0.5	0.1667	0.1667
12	Drug*Time	Kerlosin	1	0.1667	0.1667
13	Drug*Time	Kerlosin	1.5	0.1667	0.1667
14	Drug*Time	Kerlosin	2	0.1667	0.1667
15	Drug*Time	Kerlosin	2.5	0.1667	0.1667
16	Drug*Time	Kerlosin	3	0.1667	0.1667
17	Drug*Time	Laposec	0.5	-0.1667	
18	Drug*Time	Laposec	1	-0.1667	
19	Drug*Time	Laposec	1.5	-0.1667	
20	Drug*Time	Laposec	2	-0.1667	
21	Drug*Time	Laposec	2.5	-0.1667	
22	Drug*Time	Laposec	3	-0.1667	
23	Drug*Time	Placebo	0.5		-0.1667
24	Drug*Time	Placebo	1		-0.1667
25	Drug*Time	Placebo	1.5		-0.1667
26	Drug*Time	Placebo	2		-0.1667
27	Drug*Time	Placebo	2.5		-0.1667
28	Drug*Time	Placebo	3		-0.1667

### L Matrix for Testing a Covariate in a One-Factor (3 levels) Model with a Covariate

No.	Effect	Drug	L1
1	Intercept		
2	Drug	Kerlosin	
3	Drug	Laposec	
4	Drug	Placebo	
5	Weight		1.0000
6	Drug*Weight	Kerlosin	0.3333
7	Drug*Weight	Laposec	0.3333
8	Drug*Weight	Placebo	0.3333

## Kenward and Roger Fixed Effects Hypothesis Tests

Hypothesis tests have the general form

$$H_0: \mathbf{L}\boldsymbol{\beta} = \mathbf{0}$$

where  $\mathbf{L}$  is a linear contrast matrix of rank  $h$  corresponding to the desired comparisons to be made in the hypothesis test. Let  $d$  be the denominator degrees of freedom and  $q$  be the number of variance-covariance parameters, which is the dimension of  $\mathbf{W}$  (defined below).

The Kenward and Roger (1997) test statistic for testing  $H_0$  is

$$\mathbf{F}_{h,d} = \frac{\lambda}{h} \widehat{\boldsymbol{\beta}}' \mathbf{L}' (\mathbf{L}\mathbf{C}^* \mathbf{L}')^{-1} \mathbf{L} \widehat{\boldsymbol{\beta}}$$

where

$$\mathbf{C}^* = \mathbf{C} + 2\mathbf{C} \left\{ \sum_{r=1}^q \sum_{s=1}^q \mathbf{W}_{rs} \left( \mathbf{Q}_{rs} - \mathbf{P}_r \mathbf{C} \mathbf{P}_s - \frac{1}{4} \mathbf{S}_{rs} \right) \right\} \mathbf{C}$$

$$\mathbf{C} = (\mathbf{X}' \mathbf{V}^{-1} \mathbf{X})^{-1}$$

$$\mathbf{Q}_{rs} = \mathbf{X}' \mathbf{V}^{-1} \dot{\mathbf{V}}_r \mathbf{V}^{-1} \dot{\mathbf{V}}_s \mathbf{V}^{-1} \mathbf{X} = \sum_{i=1}^N \mathbf{X}'_i \mathbf{V}_i^{-1} \dot{\mathbf{V}}_{ri} \mathbf{V}_i^{-1} \dot{\mathbf{V}}_{si} \mathbf{V}_i^{-1} \mathbf{X}_i$$

$$\mathbf{P}_r = -\mathbf{X}' \mathbf{V}^{-1} \dot{\mathbf{V}}_r \mathbf{V}^{-1} \mathbf{X} = -\sum_{i=1}^N \mathbf{X}'_i \mathbf{V}_i^{-1} \dot{\mathbf{V}}_{ri} \mathbf{V}_i^{-1} \mathbf{X}_i$$

$$\mathbf{S}_{rs} = \mathbf{X}' \mathbf{V}^{-1} \dot{\mathbf{V}}_{rs} \mathbf{V}^{-1} \mathbf{X} = \sum_{i=1}^N \mathbf{X}'_i \mathbf{V}_i^{-1} \dot{\mathbf{V}}_{rsi} \mathbf{V}_i^{-1} \mathbf{X}_i$$

$$\mathbf{W} = \mathbf{H}^{-1}$$

$$\{\mathbf{H}\}_{rs} = \{\text{Hessian}\}_{rs}$$

$$\dot{\mathbf{V}}_r = \frac{\partial \mathbf{V}}{\partial \sigma_r}$$

$$\dot{\mathbf{V}}_{rs} = \frac{\partial^2 \mathbf{V}}{\partial \sigma_r \partial \sigma_s}$$

$$\mathbf{T} = \mathbf{L}' (\mathbf{L}\mathbf{C}\mathbf{L}')^{-1} \mathbf{L}$$

$$a_1 = \sum_{r=1}^q \sum_{s=1}^q \mathbf{W}_{rs} \text{tr}(\mathbf{T}\mathbf{C}\mathbf{P}_r \mathbf{C}) \text{tr}(\mathbf{T}\mathbf{C}\mathbf{P}_s \mathbf{C})$$

$$a_2 = \sum_{r=1}^q \sum_{s=1}^q \mathbf{W}_{rs} \text{tr}(\mathbf{TCP}_r \mathbf{CTCP}_s \mathbf{C})$$

$$a_3 = \frac{a_1 + 6a_2}{2h}$$

$$e = \left(1 - \frac{a_2}{h}\right)^{-1}$$

$$v = \frac{2}{h} \left\{ \frac{1 + c_1 a_3}{(1 - c_2 a_3)^2 (1 - c_3 a_3)} \right\}$$

$$c_1 = \frac{g}{3h + 2(1 - g)}$$

$$c_2 = \frac{h - g}{3h + 2(1 - g)}$$

$$c_3 = \frac{h + 2 - g}{3h + 2(1 - g)}$$

$$c_4 = \frac{v}{2e^2}$$

$$g = \frac{(h + 1)a_1 - (h + 4)a_2}{(h + 2)a_2}$$

$$d = 4 + \frac{h + 2}{c_4 h - 1}$$

$$\lambda = \frac{d}{e(d - 2)}$$

---

## Kenward and Roger Fixed Effects Confidence Intervals

Confidence intervals for linear combinations of  $\boldsymbol{\beta}$  are formed as

$$\mathbf{L}\hat{\boldsymbol{\beta}} \pm \mathbf{t}_{m,\alpha/2} \sqrt{\mathbf{LCL}'}$$

where  $\mathbf{t}_{m,\alpha/2}$  is the  $1 - \alpha/2$  percentile of the  $t$  distribution with  $m$  degrees of freedom, with  $\mathbf{C}$ ,  $\mathbf{L}$ , and  $m$  defined above.

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## Solution Algorithms

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### Methods for Finding Likelihood Solutions (Newton-Raphson, Fisher Scoring, MIVQUE, and Differential Evolution)

There are four techniques in the Mixed Models procedure for determining the maximum likelihood or restricted maximum likelihood solution (optimum): Newton-Raphson, Fisher Scoring, MIVQUE, and Differential Evolution.

The general steps for the Newton-Raphson, Fisher Scoring, and Differential Evolution techniques are (let  $\theta$  be the overall covariance parameter vector):

1. Roughly estimate  $\theta$  according to the specified structure for each.
2. Evaluate the likelihood of the model given the data and the estimates of  $\theta$ .
3. Improve upon the estimates of  $\theta$  using a search algorithm. (Improvement is defined as an increase in likelihood.)
4. Iterate until maximum likelihood is reached, according to some convergence criterion.
5. Use the final  $\theta$  estimates to estimate  $\beta$ .

#### Newton-Raphson and Fisher Scoring

The differences in the techniques revolve around the initial estimates in Step 1, and the improvements in estimates made in Step 3. For the Newton-Raphson and Fisher Scoring techniques, Step 3 occurs as follows:

- 3a. With the estimated  $\theta$ , compute the gradient vector  $\mathbf{g}$ , and the Hessian matrix  $\mathbf{H}$ .
- 3b. Compute  $\mathbf{d} = -\mathbf{H}^{-1}\mathbf{g}$ .
- 3c. Let  $\lambda = 1$ .
- 3d. Compute new estimates for  $\theta$ , iteratively, using  $\theta_i = \theta_{i-1} + \lambda\mathbf{d}$ .
- 3e. If  $\theta_i$  is a valid set of covariance parameters and improves the likelihood, continue to 3f. Otherwise, reduce  $\lambda$  by half and return to Step 3d.
- 3f. Check for convergence. If the convergence criteria (small change in  $-2\log$ -likelihood) are met, stop. If the convergence criteria are not met, go back to Step 3a.

The gradient vector  $\mathbf{g}$ , and the Hessian matrix  $\mathbf{H}$ , used for the Newton-Raphson and Fisher Scoring techniques for solving the REML equations are shown in the following table:

**REML Gradient (g) and Hessian (H)**

Technique	Gradient (g)	Hessian (H)
Newton-Raphson	$\mathbf{g}_1 + \mathbf{g}_2 + \mathbf{g}_3$	$\mathbf{H}_1 + \mathbf{H}_2 + \mathbf{H}_3$
Fisher Scoring	$\mathbf{g}_1 + \mathbf{g}_2 + \mathbf{g}_3$	$-\mathbf{H}_1 + \mathbf{H}_3$

The gradient vector  $\mathbf{g}$ , and the Hessian matrix  $\mathbf{H}$ , used for the Newton-Raphson and Fisher Scoring techniques for solving the ML equations are shown in the following table:

**ML Gradient (g) and Hessian (H)**

Technique	Gradient (g)	Hessian (H)
Newton-Raphson	$\mathbf{g}_1 + \mathbf{g}_2$	$\mathbf{H}_1 + \mathbf{H}_2$
Fisher Scoring	$\mathbf{g}_1 + \mathbf{g}_2$	$-\mathbf{H}_1$

where  $\mathbf{g}_1, \mathbf{g}_2, \mathbf{g}_3, \mathbf{H}_1, \mathbf{H}_2,$  and  $\mathbf{H}_3$  are defined as in Wolfinger, Tobias, and Sall (1994).

**Definitions**

$$\dot{\mathbf{V}}_{ri} = \frac{\partial \mathbf{V}_i}{\partial \sigma_r}, \quad \ddot{\mathbf{V}}_{rsi} = \frac{\partial^2 \mathbf{V}_i}{\partial \sigma_r \partial \sigma_s}, \quad \mathbf{e}_i = \mathbf{y}_i - \mathbf{X}_i \boldsymbol{\beta}, \quad \mathbf{A}_i = \mathbf{X}'_i \mathbf{V}_i^{-1} \mathbf{X}_i, \quad \mathbf{A} = \sum_{i=1}^N \mathbf{X}'_i \mathbf{V}_i^{-1} \mathbf{X}_i = \sum_{i=1}^N \mathbf{A}_i, \quad \mathbf{C} = \mathbf{A}^{-1}$$

$$\dot{\mathbf{A}}_r = \sum \mathbf{X}'_i \left( \frac{\partial \mathbf{V}_i^{-1}}{\partial \sigma_r} \right) \mathbf{X}_i = - \sum \mathbf{X}'_i (\mathbf{V}_i^{-1} \dot{\mathbf{V}}_{ri} \mathbf{V}_i^{-1}) \mathbf{X}_i = -\mathbf{P}_r$$

$$\mathbf{X}^* = \mathbf{X}\mathbf{K}, \quad \mathbf{K}\mathbf{K}' = (\mathbf{X}'\mathbf{V}^{-1}\mathbf{X})^{-1}$$

**Likelihoods**

$$\mathbf{l}_1 = \frac{1}{2} \sum_{i=1}^N \ln |\mathbf{V}_i|, \quad \mathbf{l}_2 = \frac{1}{2} \sum_{i=1}^N \mathbf{e}'_i \mathbf{V}_i^{-1} \mathbf{e}_i, \quad \mathbf{l}_3 = \frac{1}{2} \ln \left| \sum_{i=1}^N \mathbf{X}'_i \mathbf{V}_i^{-1} \mathbf{X}_i \right| = \frac{1}{2} \ln \left| \sum_{i=1}^N \mathbf{A}_i \right| = \frac{1}{2} \ln |\mathbf{A}|$$

**First Derivatives**

$$\mathbf{g}_{1r} = \frac{\partial \mathbf{l}_1}{\partial \sigma_r} = \frac{1}{2} \sum_{i=1}^N \text{tr}(\mathbf{V}_i^{-1} \dot{\mathbf{V}}_{ri})$$

$$\mathbf{g}_{2r} = \frac{\partial \mathbf{l}_2}{\partial \sigma_r} = -\frac{1}{2} \sum_{i=1}^N \mathbf{e}'_i \mathbf{V}_i^{-1} \dot{\mathbf{V}}_{ri} \mathbf{V}_i^{-1} \mathbf{e}_i$$

$$\mathbf{g}_{3r} = \frac{\partial \mathbf{l}_3}{\partial \sigma_r} = -\frac{1}{2} \text{tr}[\mathbf{H}_3^r]$$

## Second Derivatives

$$\mathbf{H}_{1rs} = \frac{\partial^2 \mathbf{I}_1}{\partial \boldsymbol{\sigma}_r \partial \boldsymbol{\sigma}_s} = -\frac{1}{2} \sum_{i=1}^N \{ \text{tr}(\mathbf{V}_i^{-1} \dot{\mathbf{V}}_{rsi}) - \text{tr}(\mathbf{V}_i^{-1} \dot{\mathbf{V}}_{ri} \mathbf{V}_i^{-1} \dot{\mathbf{V}}_{si}) \}$$

$$\mathbf{H}_{2rs} = \frac{\partial^2 \mathbf{I}_2}{\partial \boldsymbol{\sigma}_r \partial \boldsymbol{\sigma}_s} = \frac{1}{2} (\mathbf{H}_2^{rs} - 2\mathbf{H}_2^{r'} \mathbf{H}_2^s)$$

$$\mathbf{H}_{3rs} = \frac{\partial^2 \mathbf{I}_3}{\partial \boldsymbol{\sigma}_r \partial \boldsymbol{\sigma}_s} = \frac{1}{2} \text{tr}(\mathbf{H}_3^{rs} - \mathbf{H}_3^r \mathbf{H}_3^s)$$

See Wolfinger, Tobias, and Sall (1994), page 1299, for details.

## MIVQUE

The MIVQUE estimates of  $\boldsymbol{\theta}$  in REML estimation are found by solving

$$-(\mathbf{H}_1 + \mathbf{H}_3)\boldsymbol{\theta} = -\mathbf{g}_2.$$

The MIVQUE estimates of  $\boldsymbol{\theta}$  in ML estimation are found by solving

$$-\mathbf{H}_1\boldsymbol{\theta} = -\mathbf{g}_2.$$

See Wolfinger, Tobias, and Sall (1994), page 1306, for details.

## Differential Evolution

The differential evolution techniques used in the Mixed Models procedure for the ML and REML optimization are described in Price, Storn, and Lampinen (2005).

## Example 1 – Longitudinal Design (One Between-Subject Factor, One Within-Subject Factor, One Covariate)

This example has two purposes:

1. Acquaint the reader with the output for all output options. In only this example, each heading of each section of the output is described in detail.
2. Describe a typical analysis of a longitudinal design. A portion of this example involves the comparison of options for the Repeated Variance Pattern. There is some discussion as the output is presented and annotated, with a fuller discussion of model refinement and covariance options at the end of this example.

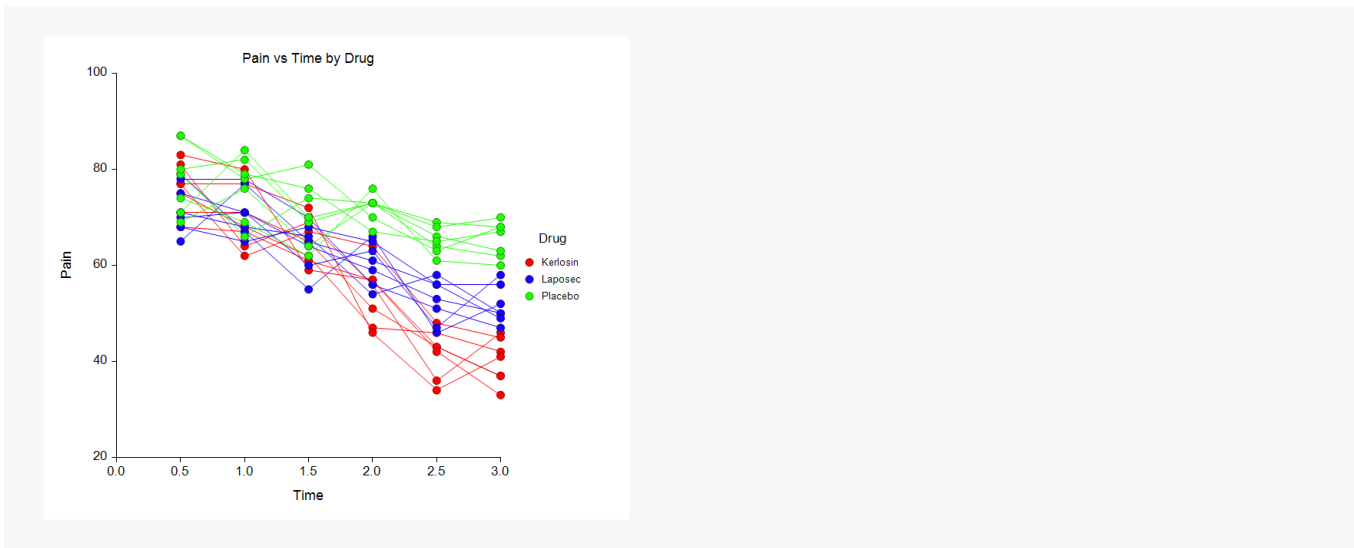
In a longitudinal design, subjects are measured more than once, usually over time. This example presents the analysis of a longitudinal design in which there is one between-subjects factor, one within-subjects factor (Time), and a covariate. Two drugs (Kerlosin and Laposec) are compared to a placebo for their effectiveness in reducing pain following a surgical eye procedure. A standard pain measurement for each patient is measured at 30 minute intervals following surgery and administration of the drug (or placebo). Six measurements, with the last at Time = 3 hours, are made for each of the 21 patients (7 per group). A blood pressure measurement of each individual at the time of pain measurement is measured as a covariate. The researchers wish to compare the drugs at the covariate value of 140.

### Pain Dataset

Drug	Patient	Time	Cov	Pain
Kerlosin	1	0.5	125	68
Kerlosin	1	1	196	67
Kerlosin	1	1.5	189	61
Kerlosin	1	2	135	57
Kerlosin	1	2.5	128	43
Kerlosin	1	3	151	37
Kerlosin	2	0.5	215	75
Kerlosin	2	1	151	68
Kerlosin	2	1.5	191	62
Kerlosin	2	2	212	47
Kerlosin	2	2.5	127	46
Kerlosin	2	3	133	42
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
Placebo	21	2	129	73
Placebo	21	2.5	216	68
Placebo	21	3	158	70



The following plot shows the relationship among all variables except the covariate.



## Setup

To run this example, complete the following steps:

### 1 Open the Pain example dataset

- From the File menu of the NCSS Data window, select **Open Example Data**.
- Select **Pain** and click **OK**.

### 2 Specify the Mixed Models – General procedure options

- Find and open the **Mixed Models – General** procedure using the menus or the Procedure Navigator.
- The settings for this example are listed below and are stored in the **Example 1** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

#### Variables Tab

Response Variable ..... **Pain**  
 Subject Variable..... **Patient**  
 Repeated (Time) Variable..... **Time**  
 Factor (Categorical) Variables ..... **Drug,Time**  
 Covariate (Continuous) Variables ..... **Cov**  
 Fixed Effects Model ..... **Drug Time Drug\*Time Cov Drug\*Cov Time\*Cov Drug\*Time\*Cov**  
 Random Model (Subject Terms Only)..... **Patient**

#### Covariates Tab

Covariate Variable 1 ..... **Cov**  
 Compute Means at these Values..... **140**

## Mixed Models - General

## Comparisons Tab

---

Factor Variable 1 ..... **DRUG**  
 Comparison ..... **All Pairs**  
 Factor Variable 2 ..... **TIME**  
 Comparison ..... **Baseline vs Each**  
 Baseline ..... **0.5**

## Reports Tab

---

Run Summary ..... **Checked**  
 Variance Estimates ..... **Checked**  
 Hypothesis Tests ..... **Checked**  
 L Matrices - Terms ..... **Checked**  
 Comparisons by Fixed Effects ..... **Checked**  
 Comparisons by Covariate Values ..... **Checked**  
 L Matrices - Comparisons ..... **Unchecked**  
 Means by Fixed Effects ..... **Checked**  
 Means by Covariate Values ..... **Checked**  
 L Matrices - LS Means ..... **Unchecked**  
 Fixed Effects Solution ..... **Checked**  
 Asymptotic VC Matrix ..... **Checked**  
 Vi Matrices (1st 3 Subjects) ..... **Checked**  
 Hessian Matrix ..... **Checked**

**3 Run the procedure**

- Click the **Run** button to perform the calculations and generate the output.

**Run Summary Section****Run Summary Section**


---

<b>Item</b>	<b>Value</b>
Likelihood Type	Restricted Maximum Likelihood
Fixed Model	DRUG+TIME+COV+DRUG*TIME+DRUG*COV+TIME*COV+ DRUG*TIME*COV
Random Model	PATIENT
Repeated Pattern	Diagonal
Number of Rows	126
Number of Subjects	21
Solution Type	Newton-Raphson
Fisher Iterations	3 of a possible 5
Newton Iterations	1 of a possible 40
Max Retries	10
Lambda	1
Log-Likelihood	-369.1552
-2 Log-Likelihood	738.3104
AIC (Smaller Better)	742.3104

## Mixed Models - General

Convergence	Normal
Run Time (Seconds)	0.867

---

This section provides a summary of the model and the iterations toward the maximum log likelihood.

### Likelihood Type

This value indicates that restricted maximum likelihood was used rather than maximum likelihood.

### Fixed Model

The model shown is that entered as the Fixed Factors Model of the Variables tab. The model includes fixed terms and covariates.

### Random Model

The model shown is that entered as the Random Factors Model of the Variables tab.

### Repeated Model

The pattern shown is that entered as the Repeated (Time) Variance Pattern of the Variables tab.

### Number of Rows

The number of rows processed from the database.

### Number of Subjects

The number of unique subjects from the database.

### Solution Type

The solution type is method used for finding the maximum (restricted) maximum likelihood solution. Newton-Raphson is the recommended method.

### Fisher Iterations

Some Fisher-Scoring iterations are used as part of the Newton-Raphson algorithm. The '4 of a possible 10' means four Fisher-Scoring iterations were used, while ten was the maximum that were allowed (as specified on the Maximization tab).

### Newton Iterations

The '1 of a possible 40' means one Newton-Raphson iteration was used, while forty was the maximum allowed (as specified on the Maximization tab).

### Max Retries

The maximum number of times that lambda was changed and new variance-covariance parameters found during an iteration was ten. If the values of the parameters result in a negative variance, lambda is divided by two and new parameters are generated. This process continues until a positive variance occurs or until Max Retries is reached.

## Lambda

Lambda is a parameter used in the Newton-Raphson process to specify the amount of change in parameter estimates between iterations. One is generally an appropriate selection. When convergence problems occur, reset this to 0.5.

If the values of the parameters result in a negative variance, lambda is divided by two and new parameters are generated. This process continues until a positive variance occurs or until Max Retries is reached.

## Log Likelihood

This is the log of the likelihood of the data given the variance-covariance parameter estimates. When a maximum is reached, the algorithm converges.

## -2 Log Likelihood

This is minus 2 times the log of the likelihood. When a minimum is reached, the algorithm converges.

## AIC

The Akaike Information Criterion is used for comparing covariance structures in models. It gives a penalty for increasing the number of covariance parameters in the model.

## Convergence

'Normal' convergence indicates that convergence was reached before the limit.

## Run Time (Seconds)

The run time is the amount of time used to solve the problem and generate the output.

---

## Random Component Parameter Estimates (G Matrix)

### Random Component Parameter Estimates (G Matrix)

Component Number	Parameter Number	Estimated Value	Model Term
1	1	1.6343	Patient

This section gives the random component estimates according to the Random Factors Model specifications of the Variables tab.

## Component Number

A number is assigned to each random component. The first component is the one specified on the variables tab. Components 2-5 are specified on the More Models tab.

## Parameter Number

When the random component model results in more than one parameter for the component, the parameter number identifies parameters within the component.

**Estimated Value**

The estimated value 1.6343 is the estimated patient variance component.

**Model Term**

Patient is the name of the random term being estimated.

---

## Repeated Component Parameter Estimates (R Matrix)

### Repeated Component Parameter Estimates (R Matrix)

Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	23.5867	Diagonal (Variance)

This section gives the repeated component estimates according to the Repeated Variance Pattern specifications of the Variables tab.

**Component Number**

A number is assigned to each repeated component. The first component is the one specified on the variables tab. Components 2-5 are specified on the More Models tab.

**Parameter Number**

When the repeated pattern results in more than one parameter for the component, the parameter number identifies parameters within the component.

**Estimated Value**

The estimated value 23.5867 is the estimated residual (error) variance.

**Parameter Type**

The parameter type describes the structure of the R matrix that is estimated, and is specified by the Repeated Component Pattern of the Variables tab.

## Term-by-Term Hypothesis Test Results

### Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Drug	1.8217	2	89.0	0.167729
Time	0.9780	5	88.4	0.435775
Cov	3.3021	1	87.1	0.072631
Drug*Time	0.8627	10	87.0	0.570795
Drug*Cov	0.7698	2	86.8	0.466228
Time*Cov	1.2171	5	88.5	0.307773
Drug*Time*Cov	1.0691	10	87.0	0.394694

These F-Values test Type-III (adjusted last) hypotheses.

This section contains a F-test for each component of the Fixed Component Model according to the methods described by Kenward and Roger (1997).

### Model Term

This is the name of the term in the model.

### F-Value

The F-Value corresponds to the L matrix used for testing this term in the model. The F-Value is based on the F approximation described in Kenward and Roger (1997).

### Num DF

This is the numerator degrees of freedom for the corresponding term.

### Denom DF

This is the approximate denominator degrees of freedom for this comparison as described in Kenward and Roger (1997).

### Prob Level

The Probability Level (or P-value) gives the strength of evidence (smaller Prob Level implies more evidence) that a term in the model has differences among its levels, or a slope different from zero in the case of covariate. It is the probability of obtaining the corresponding F-Value (or greater) if the null hypothesis of equal means (or no slope) is true.

## Individual Comparison Hypothesis Test Results

### Individual Comparison Hypothesis Test Results by Covariate Values

Covariates: Cov = 140.0000

Comparison/ Covariate(s)	Comparison Mean Difference	F-Value	Num DF	Denom DF	Raw Prob Level	Bonferroni Prob Level
<b>Drug</b>						
Drug		43.1757	2	32.8	0.000000	
Drug: Kerlosin - Laposec	-3.4655	4.2279	1	37.7	0.046734	0.140202 [3]
Drug: Kerlosin - Placebo	-13.5972	78.7500	1	28.9	0.000000	0.000000 [3]
Drug: Laposec - Placebo	-10.1316	39.4701	1	33.8	0.000000	0.000001 [3]
<b>Time</b>						
Time		46.5094	5	82.3	0.000000	
Time: 0.5 - 1	2.8138	1.3602	1	87.0	0.246695	1.000000 [5]
Time: 0.5 - 1.5	8.1935	20.2334	1	82.7	0.000022	0.000111 [5]
Time: 0.5 - 2	11.2966	29.2190	1	79.6	0.000001	0.000003 [5]
Time: 0.5 - 2.5	21.2629	122.1178	1	83.6	0.000000	0.000000 [5]
Time: 0.5 - 3	22.2608	152.6565	1	81.0	0.000000	0.000000 [5]
<b>Drug*Time</b>						
Drug*Time		5.3797	10	81.1	0.000005	
Drug = Kerlosin, Time: 0.5 - 1	7.0448	3.6965	1	86.3	0.057827	0.867407 [15]
Drug = Kerlosin, Time: 0.5 - 1.5	10.0459	9.4516	1	84.9	0.002837	0.042551 [15]
Drug = Kerlosin, Time: 0.5 - 2	19.7016	19.5674	1	77.8	0.000031	0.000467 [15]
Drug = Kerlosin, Time: 0.5 - 2.5	34.2896	118.8079	1	80.8	0.000000	0.000000 [15]
Drug = Kerlosin, Time: 0.5 - 3	34.7612	116.1114	1	84.3	0.000000	0.000000 [15]
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.

These F-Values test Type-III (adjusted last) hypotheses.

This section shows the F-tests for comparisons of the levels of the fixed terms of the model according to the methods described by Kenward and Roger (1997). The individual comparisons are grouped into subsets of the fixed model terms.

### Comparison/Covariate(s)

This is the comparison being made. The first line is 'Drug'. On this line, the levels of drug are compared when the covariate is equal to 140. The second line is 'Drug: Placebo - Kerlosin'. On this line, Kerlosin is compared to Placebo when the covariate is equal to 140.

### Comparison Mean Difference

This is the difference in the least squares means for each comparison.

### F-Value

The F-Value corresponds to the L matrix used for testing this comparison. The F-Value is based on the F approximation described in Kenward and Roger (1997).

### Num DF

This is the numerator degrees of freedom for this comparison.

### Denom DF

This is the approximate denominator degrees of freedom for this comparison as described in Kenward and Roger (1997).

### Raw Prob Level

The Raw Probability Level (or Raw P-value) gives the strength of evidence for a single comparison, unadjusted for multiple testing. It is the single test probability of obtaining the corresponding difference if the null hypothesis of equal means is true.

### Bonferroni Prob Level

The Bonferroni Prob Level is adjusted for multiple tests. The number of tests adjusted for is enclosed in brackets following each Bonferroni Prob Level. For example, 0.8674 [15] signifies that the probability the means are equal, given the data, is 0.8674, after adjusting for 15 tests.

---

## Least Squares (Adjusted) Means

### Least Squares (Adjusted) Means by Covariate Values

Covariates: Cov = 140.0000

Name	Mean	Standard Error of Mean	95.0% Lower Conf. Limit for Mean	95.0% Upper Conf. Limit for Mean	DF
<b>Intercept</b>					
Intercept	64.1132	0.6578	62.7756	65.4508	33.5
<b>Drug</b>					
Kerlosin	58.4256	1.1375	56.1112	60.7400	32.9
Laposec	61.8912	1.2437	59.3819	64.4005	42.3
Placebo	72.0228	1.0266	69.9077	74.1380	24.8
<b>Time</b>					
0.5	75.0845	1.3928	72.3172	77.8517	89.8
1	72.2707	1.9886	68.3199	76.2216	89.9
1.5	66.8910	1.2243	64.4584	69.3235	89.3
2	63.7879	1.6286	60.5523	67.0235	90.0
2.5	53.8216	1.3744	51.0909	56.5522	89.7
3	52.8237	1.2048	50.4299	55.2175	89.2
<b>Drug*Time</b>					
Kerlosin, 0.5	76.0661	2.5993	70.9021	81.2302	89.8
Kerlosin, 1	69.0213	2.6391	63.7782	74.2644	90.0
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.

This section gives the adjusted means for the levels of each fixed factor when Cov = 140.



## Mixed Models - General

**Name**

This is the level of the fixed term that is estimated on the line.

**Mean**

The mean is the estimated least squares (adjusted or marginal) mean at the specified value of the covariate.

**Standard Error of Mean**

This is the standard error of the mean.

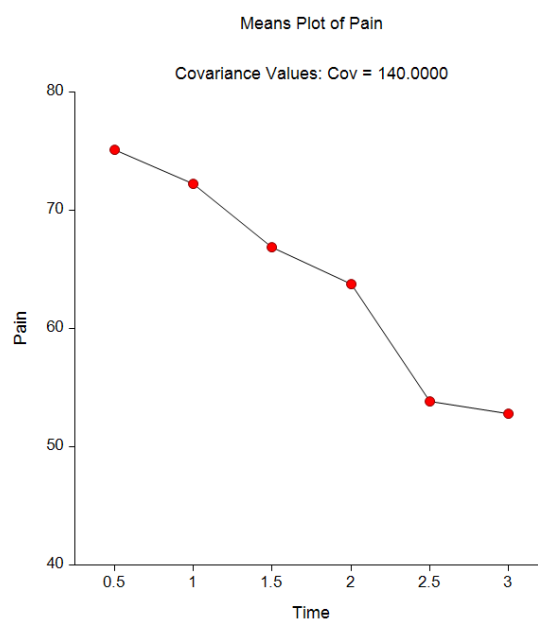
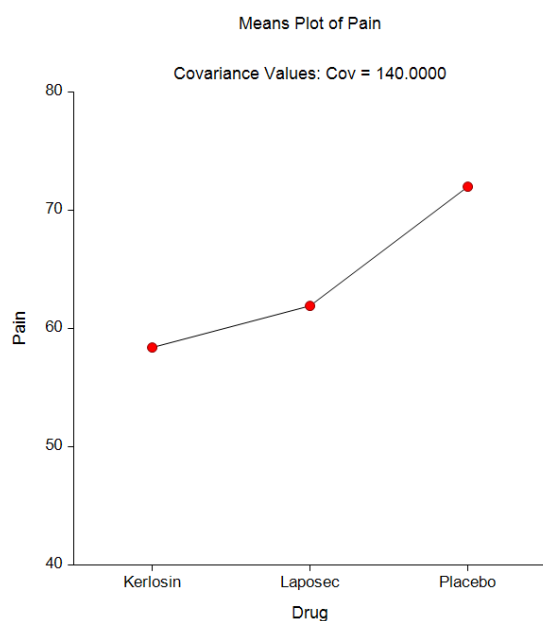
**95.0% Lower (Upper) Conf. Limit for Mean**

These limits give a 95% confidence interval for the mean.

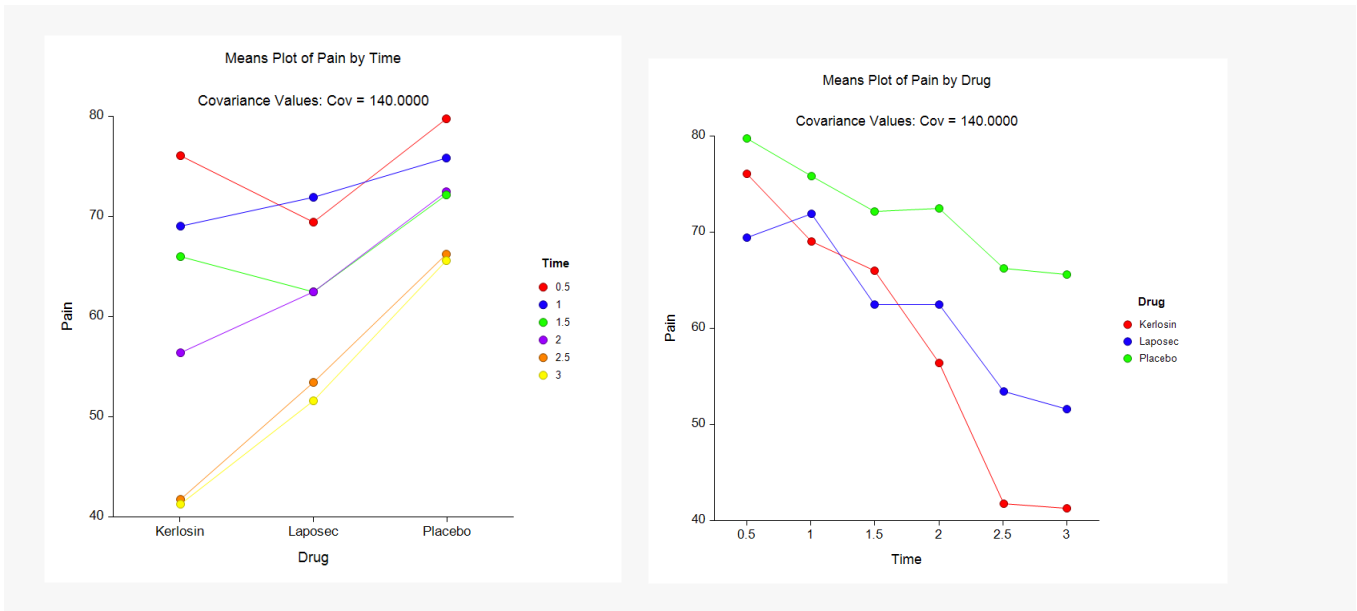
**DF**

The degrees of freedom used for the confidence limits are calculated using the method of Kenward and Roger (1997).

---

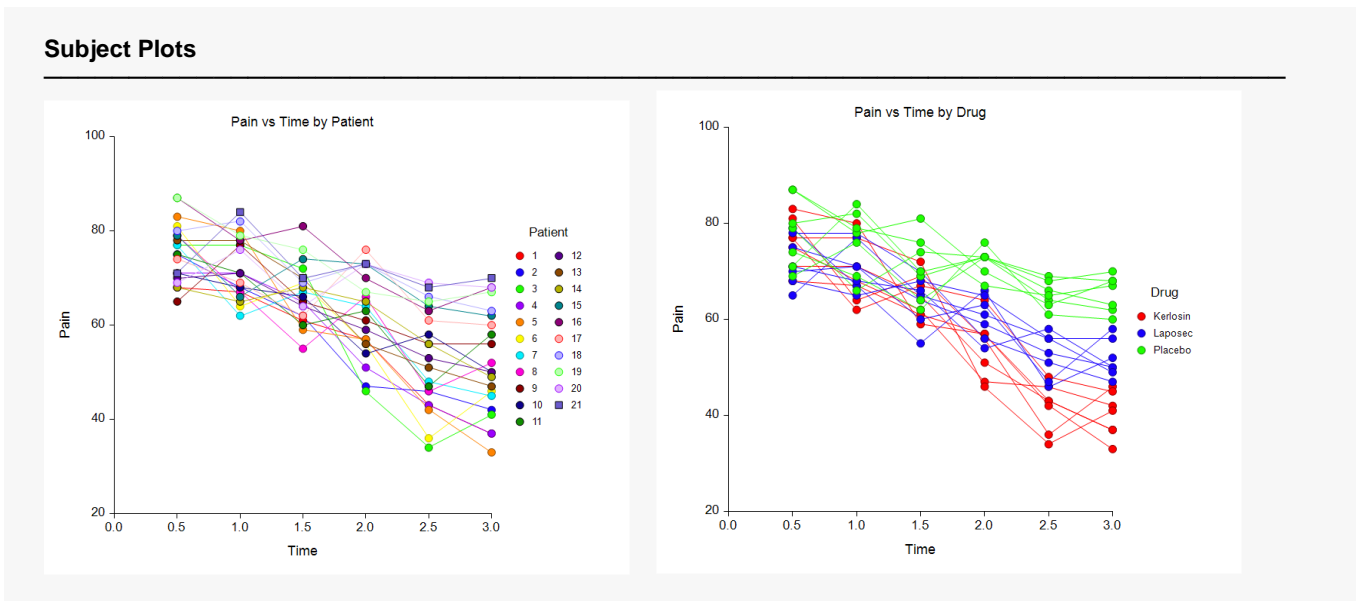
**Means Plots****Means Plots**

Mixed Models - General

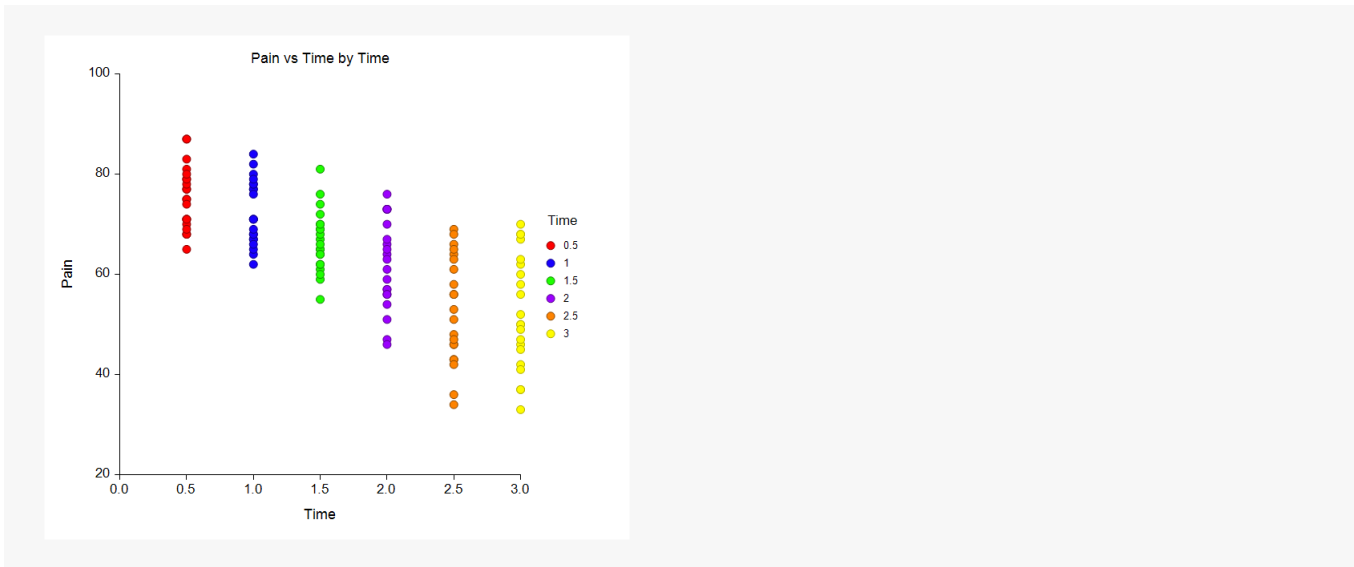


These plots show the means broken up into the categories of the fixed effects of the model. Some general trends that can be seen are those of pain decreasing with time and lower pain for the two drugs after two hours.

Subject Plots



Mixed Models - General



Each set of connected dots of the Subject plots show the repeated measurements on the same subject. The second plot is perhaps the most telling, as it shows a separation of pain among drugs after 2 hours.

## Solution for Fixed Effects

### Solution for Fixed Effects

Effect Name	Effect Estimate (Beta)	Effect Standard Error	Prob Level	95.0% Lower Conf. Limit of Beta	95.0% Upper Conf. Limit of Beta	DF	Effect No.
Intercept	66.8296	7.4693	0.000000	51.9900	81.6692	89.8	1
(Drug="Kerlosin")	-9.4849	11.9162	0.428154	-33.1595	14.1898	89.7	2
(Drug="Laposec")	-16.5164	14.6176	0.261539	-45.5591	12.5264	89.5	3
(Drug="Placebo")	0.0000	0.0000					4
(Time=0.5)	23.0382	12.0137	0.058369	-0.8336	46.9099	88.8	5
(Time=1)	-4.9520	10.5394	0.639641	-25.9029	15.9988	86.2	6
(Time=1.5)	16.5033	12.3512	0.184968	-8.0451	41.0518	87.2	7
(Time=2)	10.8739	14.7800	0.463980	-18.5236	40.2714	82.9	8
(Time=2.5)	3.0828	12.5528	0.806621	-21.8933	28.0589	81.0	9
(Time=3)	0.0000	0.0000					10
Cov	-0.0089	0.0461	0.846662	-0.1004	0.0826	89.6	11
(Drug="Kerlosin")*(Time=0.5)	-4.0793	17.5910	0.817159	-39.0401	30.8815	87.6	12
(Drug="Kerlosin")*(Time=1)	12.5535	17.5889	0.477371	-22.4218	47.5288	84.4	13
(Drug="Kerlosin")*(Time=1.5)	0.5047	16.9396	0.976299	-33.1511	34.1604	89.6	14
(Drug="Kerlosin")*(Time=2)	-4.3550	22.4012	0.846297	-48.8635	40.1535	89.3	15
(Drug="Kerlosin")*(Time=2.5)	1.7025	20.5500	0.934174	-39.1709	42.5758	83.0	16
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.

This section shows the model estimates for all the model terms (betas).

### Effect No.

This number identifies the effect of the line.

**Effect Name**

The Effect Name is the level of the fixed effect that is examine on the line.

**Effect Estimate (Beta)**

The Effect Estimate is the beta-coefficient for this effect of the model. For main effects terms the number of effects per term is the number of levels minus one. An effect estimate of zero is given for the last effect(s) of each term. There may be several zero estimates for effects of interaction terms.

**Effect Standard Error**

This is the standard error for the corresponding effect.

**Prob Level**

The Prob Level tests whether the effect is zero.

**95.0% Lower (Upper) Conf. Limit of Beta**

These limits give a 95% confidence interval for the effect.

**DF**

The degrees of freedom used for the confidence limits and hypothesis tests are calculated using the method of Kenward and Roger (1997).

---

## Asymptotic Variance-Covariance Matrix of Variance Estimates

### Asymptotic Variance-Covariance Matrix of Variance Estimates

Parameter	G(1,1)	R(1,1)
G(1,1)	4.5645	-2.6362
R(1,1)	-2.6362	15.0707

This section gives the asymptotic variance-covariance matrix of the variance components of the model. Here, the variance of the Patient variance component is 4.5645. The variance of the residual variance is 15.0707.

**Parm**

Parm is the heading for both the row variance parameters and column variance parameters.

**G(1,1)**

The two elements of G(1,1) refer to the component number and parameter number of the covariance parameter in G.

**R(1,1)**

The two elements of R(1,1) refer to the component number and parameter number of the covariance parameter in R.

## Estimated Vi Matrix of Subject = X

**Estimated Vi Matrix of Subject = 1**

Vi	1	2	3	4	5	6
1	25.2210	1.6343	1.6343	1.6343	1.6343	1.6343
2	1.6343	25.2210	1.6343	1.6343	1.6343	1.6343
3	1.6343	1.6343	25.2210	1.6343	1.6343	1.6343
4	1.6343	1.6343	1.6343	25.2210	1.6343	1.6343
5	1.6343	1.6343	1.6343	1.6343	25.2210	1.6343
6	1.6343	1.6343	1.6343	1.6343	1.6343	25.2210

**Estimated Vi Matrix of Subject = 2**

Vi	1	2	3	4	5	6
1	25.2210	1.6343	1.6343	1.6343	1.6343	1.6343
2	1.6343	25.2210	1.6343	1.6343	1.6343	1.6343
3	1.6343	1.6343	25.2210	1.6343	1.6343	1.6343
4	1.6343	1.6343	1.6343	25.2210	1.6343	1.6343
5	1.6343	1.6343	1.6343	1.6343	25.2210	1.6343
6	1.6343	1.6343	1.6343	1.6343	1.6343	25.2210

**Estimated Vi Matrix of Subject = 3**

Vi	1	2	3	4	5	6
1	25.2210	1.6343	1.6343	1.6343	1.6343	1.6343
2	1.6343	25.2210	1.6343	1.6343	1.6343	1.6343
3	1.6343	1.6343	25.2210	1.6343	1.6343	1.6343
4	1.6343	1.6343	1.6343	25.2210	1.6343	1.6343
5	1.6343	1.6343	1.6343	1.6343	25.2210	1.6343
6	1.6343	1.6343	1.6343	1.6343	1.6343	25.2210

This section gives the estimated variance-covariance matrix for each of the first three subjects.

### 1 - 6

Each of the 6 levels shown here represents one of the time values. That is 1 is for 0.5 hours, 2 is for 1 hour, 3 is for 1.5 hours, and so on. The number 25.2210 is calculated by adding the two variance estimates together,  $1.6343 + 23.5867 = 25.2210$ .

## Hessian Matrix of Variance Estimates

### Hessian Matrix of Variance Estimates

Parameter	G(1,1)	R(1,1)
G(1,1)	0.2437	0.0426
R(1,1)	0.0426	0.0738

The Hessian Matrix is directly related to the asymptotic variance-covariance matrix of the variance estimates.

### Parm

Parm is the heading for both the row variance parameters and column variance parameters.

### G(1,1)

The two elements of G(1,1) refer to the component number and parameter number of the covariance parameter in G.

### R(1,1)

The two elements of R(1,1) refer to the component number and parameter number of the covariance parameter in R.

## L Matrices

### L Matrix for Drug

No.	Effect	Drug	Time	L1	L2
1	Intercept				
2	Drug	Kerlosin		1.0000	1.0000
3	Drug	Laposec		-1.0000	
4	Drug	Placebo			-1.0000
5	Time		0.5		
6	Time		1		
7	Time		1.5		
8	Time		2		
9	Time		2.5		
10	Time		3		
11	Cov				
12	Drug*Time	Kerlosin	0.5	0.1667	0.1667
13	Drug*Time	Kerlosin	1	0.1667	0.1667
14	Drug*Time	Kerlosin	1.5	0.1667	0.1667
15	Drug*Time	Kerlosin	2	0.1667	0.1667
16	Drug*Time	Kerlosin	2.5	0.1667	0.1667
17	Drug*Time	Kerlosin	3	0.1667	0.1667
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.

(report continues with several pages of output)

## Mixed Models - General

The L matrices are used to form a linear combination of the betas corresponding to a specific hypothesis test or mean estimate. The L matrix in this example is used for testing whether there is a difference among the three levels of Drug.

**No.**

This number is used for identifying the corresponding beta term.

**Effect**

This column gives the model term.

**Factor Variables (e.g. Drug, Time)**

These columns identify the level of each fixed effect to which the coefficients of the L matrix of the same line correspond.

**L1, L2, L3, ...**

L1, L2, L3, ... are a group of column vectors that combine to form an L matrix. The L matrix in this example is used for testing whether there is a difference among the three levels of Drug.

---

## Discussion of Example 1 Results

The output shown for this example to this point has been for the full model with all interactions. It has been shown to illustrate the several sections of output that are available. In practice, when dealing with covariates, this model should be refined before making conclusions concerning the two drugs in question. The original F-test results are repeated below.

### Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Drug	1.8217	2	89.0	0.167729
Time	0.9780	5	88.4	0.435775
Cov	3.3021	1	87.1	0.072631
Drug*Time	0.8627	10	87.0	0.570795
Drug*Cov	0.7698	2	86.8	0.466228
Time*Cov	1.2171	5	88.5	0.307773
Drug*Time*Cov	1.0691	10	87.0	0.394694

These F-Values test Type-III (adjusted last) hypotheses.

Using a hierarchical step-down approach to model improvement, we begin by removing the highest order term, the three-way interaction (F-Value = 1.07, Prob Level = 0.3947). The F-test results for this new model are as follows:

Mixed Models - General

**Term-by-Term Hypothesis Test Results**

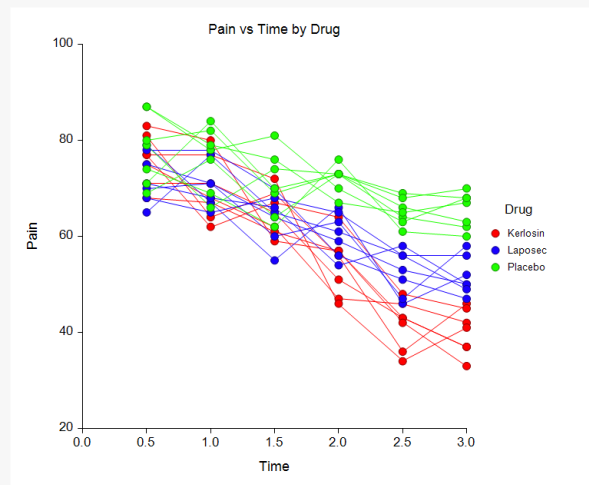
Model Term	F-Value	Num DF	Denom DF	Prob Level
Drug	4.1700	2	96.8	0.018315
Time	1.3419	5	98.0	0.253118
Cov	1.7686	1	98.9	0.186615
Drug*Time	7.4394	10	84.1	0.000000
Drug*Cov	2.2336	2	92.4	0.112895
Time*Cov	2.3667	5	98.0	0.045036

These F-Values test Type-III (adjusted last) hypotheses.

Since all interaction Prob Levels are now quite small, this model appears to be reasonable. Some researchers might argue to continue refinement by removing the Drug\*Cov interaction (F-Value = 2.23, Prob Level = 0.1129). Such an argument is also reasonable, but this is not the course that is pursued here, since a moderately low prob level indicates there may be a mild Drug\*Cov interaction effect.

The dominant prob level is the one associated with the Drug\*Time interaction (F-Value = 7.44, Prob Level = 0.0000). This interaction can be clearly seen in the following scatter plot of the individual subjects. Note that the Placebo group does not decrease as rapidly as the Kerlosin group.

**Subject Plots**



This interaction can be examined in greater detail by comparing the three levels of Drug at each time point (at the covariate value of 140).



Mixed Models - General

**Individual Comparison Hypothesis Test Results by Covariate Values**

Covariates: Cov = 140.0000

Comparison/ Covariate(s)	Comparison Mean Difference	F-Value	Num DF	Denom DF	Raw Prob Level	Bonferroni Prob Level
<b>Drug*Time</b>						
Time = 0.5, Drug: Kerlosin - Laposec	6.1619	4.3285	1	100.0	0.040036	0.720656 [18]
Time = 0.5, Drug: Kerlosin - Placebo	-1.0451	0.1287	1	100.0	0.720543	1.000000 [18]
Time = 0.5, Drug: Laposec - Placebo	-7.2070	6.3717	1	100.0	0.013169	0.237046 [18]
Time = 1, Drug: Kerlosin - Laposec	1.4659	0.2530	1	100.0	0.616068	1.000000 [18]
Time = 1, Drug: Kerlosin - Placebo	-7.2872	6.2095	1	99.9	0.014353	0.258346 [18]
Time = 1, Drug: Laposec - Placebo	-8.7531	8.1482	1	100.0	0.005241	0.094336 [18]
Time = 1.5, Drug: Kerlosin - Laposec	2.3523	0.7185	1	99.9	0.398661	1.000000 [18]
Time = 1.5, Drug: Kerlosin - Placebo	-5.2756	3.6844	1	99.8	0.057780	1.000000 [18]
Time = 1.5, Drug: Laposec - Placebo	-7.6278	7.5730	1	99.9	0.007037	0.126669 [18]
Time = 2, Drug: Kerlosin - Laposec	-2.4751	0.6341	1	100.0	0.427746	1.000000 [18]
Time = 2, Drug: Kerlosin - Placebo	-14.1184	19.4393	1	100.0	0.000026	0.000471 [18]
Time = 2, Drug: Laposec - Placebo	-11.6433	17.6388	1	99.8	0.000058	0.001046 [18]
Time = 2.5, Drug: Kerlosin - Laposec	-11.0499	16.5687	1	99.7	0.000094	0.001693 [18]
Time = 2.5, Drug: Kerlosin - Placebo	-27.1071	70.0980	1	100.0	0.000000	0.000000 [18]
Time = 2.5, Drug: Laposec - Placebo	-16.0572	26.3720	1	100.0	0.000001	0.000025 [18]
Time = 3, Drug: Kerlosin - Laposec	-10.7977	15.6495	1	99.8	0.000143	0.002569 [18]
Time = 3, Drug: Kerlosin - Placebo	-25.1938	84.9230	1	99.8	0.000000	0.000000 [18]
Time = 3, Drug: Laposec - Placebo	-14.3960	27.5367	1	99.8	0.000001	0.000016 [18]

These F-Values test Type-III (adjusted last) hypotheses.

The first Bonferroni-adjusted significant difference among levels of treatment occurs at Time = 2 hours. At Time = 2, the Kerlosin and Laposec means are significantly different from the Placebo mean (Bonferroni Prob Levels = 0.0005 and 0.0010, respectively), but not from each other (Bonferroni Prob Level = 1.0000). At times 2.5 hours and 3 hours all levels of Drug are significantly different, with Kerlosin showing the greatest pain reduction.

**Repeated and Random Component Specification**

Another issue that should be considered from the beginning of the analysis is the covariance structure of the repeated measurements over time. The specification to this point involved both random (**G**) and the repeated (**R**) components of the model. The **G** and the **R** matrices are used to form the complete variance-covariance matrix of all the responses using the formula  $V = ZGZ' + R$ . The **R** and the **R** used to this point have the form

$$G = \begin{pmatrix} \sigma_s^2 & & & \\ & \sigma_s^2 & & \\ & & \sigma_s^2 & \\ & & & \sigma_s^2 \end{pmatrix} \quad R = \begin{pmatrix} \sigma^2 & & & & \\ & \sigma^2 & & & \\ & & \sigma^2 & & \\ & & & \sigma^2 & \\ & & & & \sigma^2 \end{pmatrix}$$

where **G** has dimension 21 by 21 and R has dimension 126 by 126. The resulting variance-covariance matrix,  $V = ZGZ' + R$ , has the form

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$$\mathbf{V} = \begin{pmatrix}
 \sigma^2 + \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & 0 & 0 & \dots \\
 \sigma_S^2 & \sigma^2 + \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & 0 & 0 & \dots \\
 \sigma_S^2 & \sigma_S^2 & \sigma^2 + \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & 0 & 0 & \dots \\
 \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & \sigma^2 + \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & 0 & 0 & \dots \\
 \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & \sigma^2 + \sigma_S^2 & \sigma_S^2 & 0 & 0 & \dots \\
 \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & \sigma_S^2 & \sigma^2 + \sigma_S^2 & 0 & 0 & \dots \\
 0 & 0 & 0 & 0 & 0 & 0 & \sigma^2 + \sigma_S^2 & \sigma_S^2 & \dots \\
 0 & 0 & 0 & 0 & 0 & 0 & \sigma_S^2 & \sigma^2 + \sigma_S^2 & \dots \\
 \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \ddots
 \end{pmatrix}$$

where each 6 by 6 block corresponds to a single patient. The full dimension of this matrix is  $6 \times 21 = 126$  by 126.

The estimates of  $\sigma_S^2$  and  $\sigma^2$  for the model without the three-way interaction are 0.7066 and 24.6293, as shown in the output below.

**Random Component Parameter Estimates (G Matrix)**

Component Number	Parameter Number	Estimated Value	Model Term
1	1	0.7066	Patient

**Repeated Component Parameter Estimates (R Matrix)**

Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	24.6293	Diagonal (Variance)

The resulting 6 by 6 matrix for each subject (as shown in the output) is

**Estimated Vi Matrix of Subject = 1**

Vi	1	2	3	4	5	6
1	25.3359	0.7066	0.7066	0.7066	0.7066	0.7066
2	0.7066	25.3359	0.7066	0.7066	0.7066	0.7066
3	0.7066	0.7066	25.3359	0.7066	0.7066	0.7066
4	0.7066	0.7066	0.7066	25.3359	0.7066	0.7066
5	0.7066	0.7066	0.7066	0.7066	25.3359	0.7066
6	0.7066	0.7066	0.7066	0.7066	0.7066	25.3359

The number 25.3359 comes from adding 0.7066 and 24.6293.

### Using Compound Symmetry as the Repeated Pattern Rather than Using a Random Component

An alternative specification that yields the same results is to remove the Random Component of the Model (Patient) and change the Repeated (Time) Covariance Pattern to *Compound Symmetry* with *Force Covariances to be Positive* checked on the Maximization tab. In this case, there is no **G** matrix and the **R** matrix has the form

$$\mathbf{R} = \begin{pmatrix} \sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 \\ \rho\sigma^2 & \sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 \\ \rho\sigma^2 & \rho\sigma^2 & \sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 \\ \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \sigma^2 & \rho\sigma^2 & \rho\sigma^2 \\ \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \sigma^2 & \rho\sigma^2 \\ \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \sigma^2 \end{pmatrix}$$

The true dimension of **R** is still 126 by 126 with 21 of the above matrices along the diagonal. The Repeated Component output becomes

Repeated Component Parameter Estimates (R Matrix)			
Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	25.3358	Diagonal (Variance)
1	1	0.0279	Off-Diagonal (Correlation)

Here, the estimate of  $\sigma^2$  is 25.3358 and the estimate of  $\rho$  is 0.0279.

The V matrix now has the form

$$\mathbf{V} = \begin{pmatrix} \sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & 0 & 0 & \dots \\ \rho\sigma^2 & \sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & 0 & 0 & \dots \\ \rho\sigma^2 & \rho\sigma^2 & \sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & 0 & 0 & \dots \\ \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \sigma^2 & \rho\sigma^2 & \rho\sigma^2 & 0 & 0 & \dots \\ \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \sigma^2 & \rho\sigma^2 & 0 & 0 & \dots \\ \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \sigma^2 & 0 & 0 & \dots \\ 0 & 0 & 0 & 0 & 0 & 0 & \sigma^2 & \rho\sigma^2 & \dots \\ 0 & 0 & 0 & 0 & 0 & 0 & \rho\sigma^2 & \sigma^2 & \dots \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \ddots \end{pmatrix}$$

and the estimated block for each subject using the compound symmetry specification is

Estimated Vi Matrix of Subject = 1						
Vi	1	2	3	4	5	6
1	25.3358	0.7065	0.7065	0.7065	0.7065	0.7065
2	0.7065	25.3358	0.7065	0.7065	0.7065	0.7065
3	0.7065	0.7065	25.3358	0.7065	0.7065	0.7065
4	0.7065	0.7065	0.7065	25.3358	0.7065	0.7065
5	0.7065	0.7065	0.7065	0.7065	25.3358	0.7065
6	0.7065	0.7065	0.7065	0.7065	0.7065	25.3358

which is identical (to rounding error) to the previous result using random and repeated component specification.

### Other Repeated Patterns (AR(1))

It is natural to expect that the covariances of measurements made closer together in time are more similar than those at more distant times. Several covariance pattern structures have been developed for such cases. A complete list of the available structures in the Mixed Models procedure is given elsewhere in the chapter. Here, we will examine one of the more common structures: AR(1).

Using the AR(1) covariance pattern, there are only two parameters,  $\sigma^2$  and  $\rho$ , but the coefficient of  $\sigma^2$  decreases exponentially as observations are farther apart. The **R** matrix has the form

$$\mathbf{R} = \begin{pmatrix} \sigma^2 & \rho\sigma^2 & \rho^2\sigma^2 & \rho^3\sigma^2 & \rho^4\sigma^2 & \rho^5\sigma^2 \\ \rho\sigma^2 & \sigma^2 & \rho\sigma^2 & \rho^2\sigma^2 & \rho^3\sigma^2 & \rho^4\sigma^2 \\ \rho^2\sigma^2 & \rho\sigma^2 & \sigma^2 & \rho\sigma^2 & \rho^2\sigma^2 & \rho^3\sigma^2 \\ \rho^3\sigma^2 & \rho^2\sigma^2 & \rho\sigma^2 & \sigma^2 & \rho\sigma^2 & \rho^2\sigma^2 \\ \rho^4\sigma^2 & \rho^3\sigma^2 & \rho^2\sigma^2 & \rho\sigma^2 & \sigma^2 & \rho\sigma^2 \\ \rho^5\sigma^2 & \rho^4\sigma^2 & \rho^3\sigma^2 & \rho^2\sigma^2 & \rho\sigma^2 & \sigma^2 \end{pmatrix}$$

The true dimension of **R** is 126 by 126 with 21 of the above matrices along the diagonal.

The Repeated Component output becomes

Repeated Component Parameter Estimates (R Matrix)			
Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	25.3360	Diagonal (Variance)
1	1	0.0659	Off-Diagonal (Correlation)

Here, the estimate of  $\sigma^2$  is 25.3360 and the estimate of  $\rho$  is 0.0659.

The estimated block for each subject using the AR(1) specification is

Estimated Vi Matrix of Subject = 1						
Vi	1	2	3	4	5	6
1	25.3360	1.6696	0.1100	0.0073	0.0005	0.0000
2	1.6696	25.3360	1.6696	0.1100	0.0073	0.0005
3	0.1100	1.6696	25.3360	1.6696	0.1100	0.0073
4	0.0073	0.1100	1.6696	25.3360	1.6696	0.1100
5	0.0005	0.0073	0.1100	1.6696	25.3360	1.6696
6	0.0000	0.0005	0.0073	0.1100	1.6696	25.3360

The estimates of the covariance parameters using this formulation are closer to 0 as the time between measurements increases.

The AIC value may be used to compare the various covariance structures. The AIC value for the AR(1) specification is 725.77. The AIC value for the compound symmetry (and random component) specification is 725.94. A smaller AIC value indicates a better model. Thus, the AR(1) specification provides a slight improvement over the compound symmetry (and random component) specification.

## Example 2a – Two-Sample T-Test Assuming Equal Variance (One Between-Subject Factor, No Within-Subject Factors, No Covariates)

Examples 2a and 2b show how the use of the Mixed Models procedure gives the same results as the corresponding two-sided test in the Two-Sample T-Test procedure. Example 2c shows the extension to include a covariate in a two-sample test, which cannot be done using the Two-Sample T-Test procedure.

One of the simplest, yet very commonly used, designs is the two-group design. In this design, subjects are randomly assigned to, or randomly drawn from, one of two groups. A response is measured, and the means are compared. The common technique for analysis in this scenario is the two-sample (two-group) T-test. The data set-up for this design is two variables.

### Two Sample Dataset

Response	Treatment
141	Treatment
146	Treatment
138	Treatment
155	Treatment
134	Treatment
136	Treatment
142	Treatment
137	Treatment
.	.
.	.
.	.
190	Placebo
186	Placebo
183	Placebo
175	Placebo

Using the T-Test – Two-Sample procedure, the two groups would be compared by entering *Response* as the Response Variable and *Treatment* as the Group Variable. An excerpt of the output appears as follows.

### Output Excerpt – Two-Sample T-Test Procedure

#### Equal-Variance T-Test

Alternative Hypothesis	Mean Difference	Standard Error	T-Statistic	DF	Prob Level	Reject H0 at $\alpha = 0.05?$
$\mu_1 - \mu_2 \neq 0$	15.14474	5.763373	2.6278	33	0.01294	Yes

The equivalence of means is rejected (Prob Level = 0.01294) at the 0.05 alpha level.

## Mixed Models - General

The corresponding analysis in the Mixed Models procedure is similar, but an additional subject variable must be added. The subject variable identifies the subject to which each row belongs. When there are no repeated measurements, a subject variable may be created quickly by clicking any cell in a blank variable, selecting *Fill* from the *Edit* menu, and clicking on *Fill*.

**Two-Sample T-Test Example Dataset – Two Sample 2**

Response	Treatment	Subject
141	Treatment	1
146	Treatment	2
138	Treatment	3
155	Treatment	4
134	Treatment	5
136	Treatment	6
142	Treatment	7
137	Treatment	8
.	.	.
.	.	.
.	.	.
190	Placebo	32
186	Placebo	33
183	Placebo	34
175	Placebo	35

## Setup

To run this example, complete the following steps:

**1 Open the Two Sample 2 example dataset**

- From the File menu of the NCSS Data window, select **Open Example Data**.
- Select **Two Sample 2** and click **OK**.

**2 Specify the Mixed Models – General procedure options**

- Find and open the **Mixed Models – General** procedure using the menus or the Procedure Navigator.
- The settings for this example are listed below and are stored in the **Example 2a** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

## Variables Tab

Response Variable .....**Response**  
 Subject Variable.....**Subject**  
 Repeated (Time) Variable.....<Empty>  
 Factor (Categorical) Variables .....**Treatment**  
 Fixed Effects Model .....**Treatment**

**3 Run the procedure**

- Click the **Run** button to perform the calculations and generate the output.

---

## Mixed Models Output

### Repeated Component Parameter Estimates (R Matrix)

Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	288.5088	Diagonal (Variance)

### Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Treatment	6.9051	1	33.0	0.012941

These F-Values test Type-III (adjusted last) hypotheses.

The Prob Level (0.0129) for the Treatment term is the same as the one given by the Two-Sample T-Test procedure. The estimate of the residual variance is 288.5088, which is the square of the standard deviation from the Two-Sample T-Test procedure.

---

## Example 2b – Two-Sample T-Test Assuming Unequal Variance (One Between-Subject Factor, No Within-Subject Factors, No Covariates)

We now examine the Two Sample dataset without assuming equal variance among the two groups. The mean response is to be compared for a treatment and placebo.

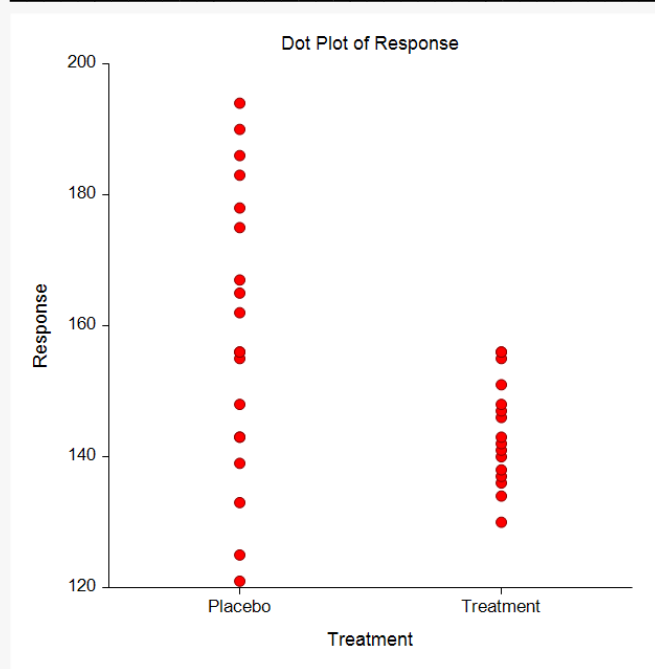
### Two Sample Dataset

Response	Treatment
141	Treatment
146	Treatment
138	Treatment
155	Treatment
134	Treatment
136	Treatment
142	Treatment
137	Treatment
.	.
.	.
.	.
190	Placebo
186	Placebo
183	Placebo
175	Placebo

The assumption of equal variance in this example is probably not a good one, as evidenced by the dot plot and equal variance tests shown below. The dot plot is obtained using the Dot Plots procedure, while the assumption tests are from the Two-Sample T-Test procedure.



**Dot Plots**



**Tests of the Equal Variance Assumption**

Equal-Variance Test	Test Statistic	Prob Level	Reject H0 of Equal Variances at $\alpha = 0.05$ ?
Variance-Ratio	7.4226	0.00030	Yes
Modified-Levene	11.8596	0.00158	Yes

The variance of the placebo group is much larger than that of the treatment group. The Aspin-Welch Unequal-Variance T-test should be used in place of the traditional T-test. An equivalent option is allowing for a separate variance for each group in the Mixed Models procedure.

Using the T-Test – Two-Sample procedure, the two groups would be compared by entering *Response* as the Response Variable and *Treatment* as the Group Variable. An excerpt of the output appears as follows.

**Unequal Variance Output Excerpt – Two-Sample T-Test Procedure**

**Aspin-Welch Unequal-Variance T-Test**

Alternative Hypothesis	Mean Difference	Standard Error	T-Statistic	DF	Prob Level	Reject H0 at $\alpha = 0.05$ ?
$\mu_1 - \mu_2 \neq 0$	15.14474	5.388208	2.8107	23.50	0.00980	Yes

The corresponding analysis in the Mixed Models procedure is similar, but an additional subject variable must be added. The subject variable identifies the subject to which each row belongs. When there are no repeated measurements, a subject variable may be created quickly by clicking any cell in a blank variable, selecting *Fill* from the *Edit* menu, and clicking on *Fill*.

**Two-Sample T-Test Example Dataset – Two Sample 2**

Response	Treatment	Subject
141	Treatment	1
146	Treatment	2
138	Treatment	3
155	Treatment	4
134	Treatment	5
136	Treatment	6
142	Treatment	7
137	Treatment	8
.	.	.
.	.	.
.	.	.
190	Placebo	32
186	Placebo	33
183	Placebo	34
175	Placebo	35

## Setup

To run this example, complete the following steps:

**1 Open the Two Sample 2 example dataset**

- From the File menu of the NCSS Data window, select **Open Example Data**.
- Select **Two Sample 2** and click **OK**.

**2 Specify the Mixed Models – General procedure options**

- Find and open the **Mixed Models – General** procedure using the menus or the Procedure Navigator.
- The settings for this example are listed below and are stored in the **Example 2b** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

## Variables Tab

Response Variable ..... **Response**  
 Subject Variable..... **Subject**  
 Repeated (Time) Variable..... **<Empty>**  
 Factor (Categorical) Variables ..... **Treatment**  
 Fixed Effects Model ..... **Treatment**  
 Repeated (Time) Covariance Pattern Groups ..... **Treatment**

**3 Run the procedure**

- Click the **Run** button to perform the calculations and generate the output.

## Mixed Models Output

### Repeated Component Parameter Estimates (R Matrix)

Component Number	Parameter Number	Group Number	Estimated Value	Parameter Type
1	1	1	475.5439	Diagonal (Variance)
1	1	2	64.0667	Diagonal (Variance)

### Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Treatment	7.9001	1	23.5	0.009801

These F-Values test Type-III (adjusted last) hypotheses.

The Prob Level (0.0098) is the same as the one given by the Aspin-Welch Unequal Variance test of the Two-Sample T-Test procedure. The two variance estimates, 475.5436 and 64.0666, correspond to the placebo and treatment groups, respectively, and are the squares of the individual group standard deviations given in the Two-Sample T-Test procedure.

Examples 2a and 2b would likely be run using the Two-Sample T-Test procedure rather than the Mixed Models procedure. These examples are provided as an introduction to running the Mixed Models procedure for a simple case, as well as to show the flexibility of the Mixed Models procedure. Example 2c shows the extension of a two-sample test to the inclusion of a covariate. The Two-Sample T-Test procedure does not permit the inclusion of a covariate.

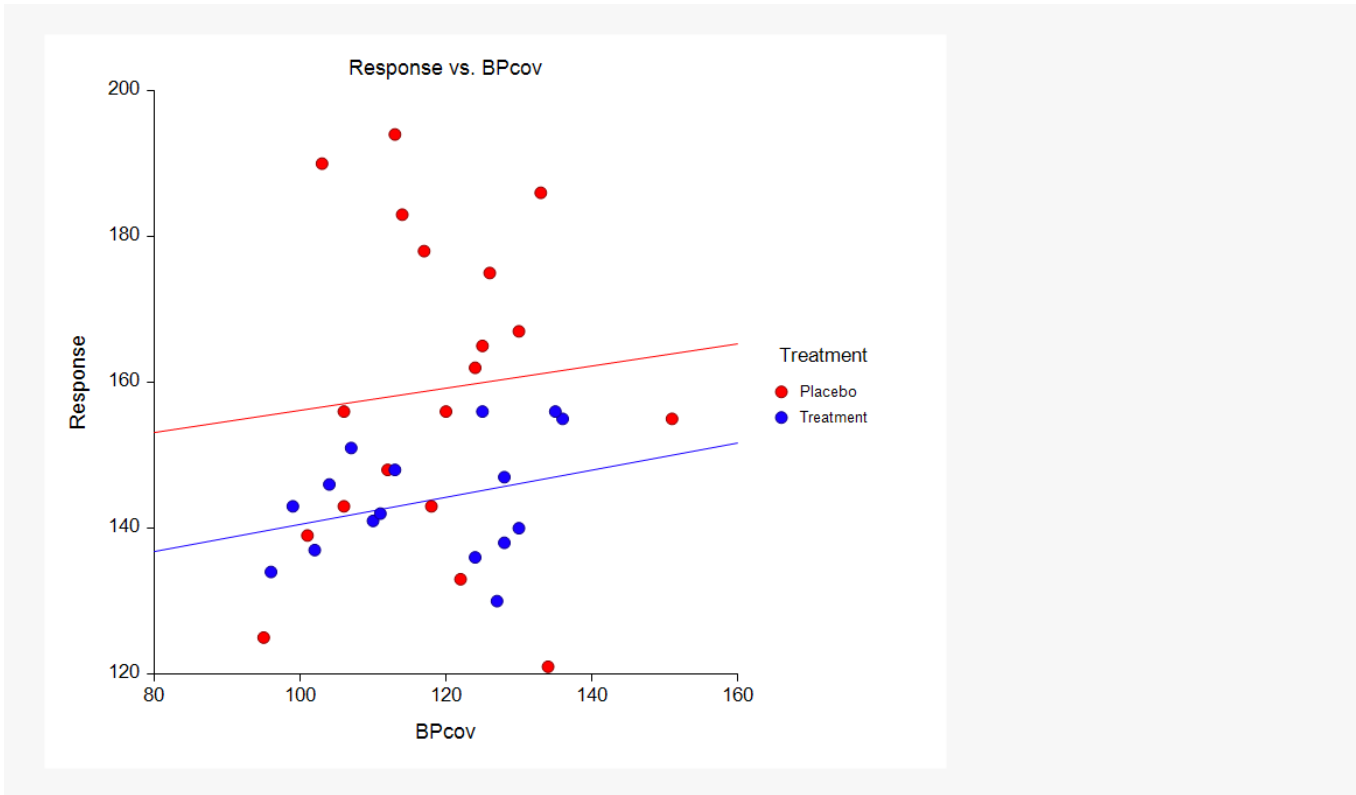
## Example 2c – Two-Sample T-Test with a Covariate (One Between-Subject Factor, No Within-Subject Factors, One Covariate)

The two-sample analyses shown in Examples 2a and 2b would likely be carried out using the T-Test – Two-Sample procedure rather than the Mixed Models procedure because the T-Test procedure is easier to use and gives more specific output. However, when a covariate is measured for each subject there is no way to incorporate this into a simple T-test. The analysis becomes analysis of covariance, or ANCOVA. The General Linear Models or Multiple Regression procedures could be used, but in those, equal variances must be assumed. The flexibility we need for this analysis can only be achieved using the Mixed Models procedure. Adding a covariate only adds a couple of steps to the analysis without a covariate. The Two Sample 2 dataset with the addition of a covariate, blood pressure (BP), becomes the Two Sample Cov dataset.

### Two Sample Cov Dataset

Response	Treatment	Subject	BPcov
141	Treatment	1	110
146	Treatment	2	104
138	Treatment	3	128
155	Treatment	4	136
134	Treatment	5	96
136	Treatment	6	124
142	Treatment	7	111
137	Treatment	8	102
.	.	.	.
.	.	.	.
.	.	.	.
190	Placebo	32	103
186	Placebo	33	133
183	Placebo	34	114
175	Placebo	35	126

A scatter plot of the two groups is obtained from the Scatter Plots procedure.



## Setup

To run this example, complete the following steps:

### 1 Open Two Sample Cov example dataset

- From the File menu of the NCSS Data window, select **Open Example Data**.
- Select **Two Sample Cov** and click **OK**.

### 2 Specify the Mixed Models - General procedure options

- Find and open the **Mixed Models - General** procedure using the menus or the Procedure Navigator.
- The settings for this example are listed below and are stored in the **Example 2c** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

#### Variables Tab

Response Variable .....	<b>Response</b>
Subject Variable.....	<b>Subject</b>
Repeated (Time) Variable.....	<b>&lt;Empty&gt;</b>
Factor (Categorical) Variables .....	<b>Treatment</b>
Covariate (Continuous) Variables.....	<b>BPCov</b>
Fixed Effects Model .....	<b>Treatment BPCov</b>
Repeated (Time) Covariance Pattern Groups .....	<b>Treatment</b>

### 3 Run the procedure

- Click the **Run** button to perform the calculations and generate the output.

## Mixed Models Output Excerpt

### Repeated Component Parameter Estimates (R Matrix)

Component Number	Parameter Number	Group Number	Estimated Value	Parameter Type
1	1	1	475.0862	Diagonal (Variance)
1	1	2	61.4904	Diagonal (Variance)

### Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Treatment	7.7098	1	23.2	0.010675
BPcov	1.5537	1	18.7	0.227938

These F-Values test Type-III (adjusted last) hypotheses.

### Least Squares (Adjusted) Means by Covariate Values

Covariates: BPcov = 117.8571

Name	Mean	Standard Error of Mean	95.0% Lower Conf. Limit for Mean	95.0% Upper Conf. Limit for Mean	DF
<b>Intercept</b>					
Intercept	151.3319	2.6855	145.7789	156.8849	23.2
<b>Treatment</b>					
Placebo	158.7928	5.0011	148.2852	169.3003	18.0
Treatment	143.8711	1.9628	139.6686	148.0736	14.3

There is not strong evidence of a relationship between BPcov and Response (Prob Level = 0.2279). The difference in Treatment levels (Placebo and Treatment) is still seen (Prob Level = 0.0107). Because BPcov has little or no effect on the Response, the variance estimates for each group are similar to those obtain with including the covariate. Least squares adjusted means are given for the mean value of the covariate.

Comparisons and/or least square means could be obtained for any value of the covariate by specifying the desired value under the Covariates tab of the Mixed Models procedure. Specifying a covariate value of BPcov = 130 gives the following output.

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**Individual Comparison Hypothesis Test Results by Covariate Values**

Covariates: BPcov = 130.0000

Comparison/ Covariate(s)	Comparison Mean Difference	F-Value	Num DF	Denom DF	Raw Prob Level	Bonferroni Prob Level
<b>Treatment</b>						
Treatment		7.7098	1	23.2	0.010675	
Treatment: Placebo - Treatment	14.9216	7.7098	1	23.2	0.010675	0.010675 [1]

These F-Values test Type-III (adjusted last) hypotheses.

**Least Squares (Adjusted) Means by Covariate Values**

Covariates: BPcov = 130.0000

Name	Mean	Standard Error of Mean	95.0% Lower Conf. Limit for Mean	95.0% Upper Conf. Limit for Mean	DF
<b>Intercept</b>					
Intercept	153.5280	3.2161	146.9675	160.0885	30.9
<b>Treatment</b>					
Placebo	160.9888	5.2751	150.0228	171.9548	21.1
Treatment	146.0672	2.7017	140.3448	151.7896	16.2

The F-test (F-Value = 7.71, Prob Level = 0.0107) is the same as the test for Treatment since the lines are assumed parallel in the fixed model. Thus, the F-test would be the same for any value of BPcov, unless the fixed model were changed to include the interaction Treatment\*BPcov.

The least squares adjusted means (160.99 and 146.07) are adjusted to the covariate value of 130.

## Example 3a – One-Way ANOVA Design Assuming Equal Variance (One Between-Subject Factor, No Within-Subject Factors, No Covariates)

In a one-way layout design, two or more (usually three or more) groups are compared. Similar to the two-sample design, one column contains the response while another column identifies the groups. In this example, four plant food mixtures (salicylic acid, low iron, high iron, and no food) are compared in their ability to promote growth in beans. Twenty-eight plots are used in the experiment. The response is the weight of the beans harvested from the plot.

### Bean Dataset

Food	Plot	Weight
Salicyl	1	256
Salicyl	2	284
Salicyl	3	255
Salicyl	4	214
Salicyl	5	283
Salicyl	6	277
Salicyl	7	263
LowIron	8	293
LowIron	9	326
LowIron	10	313
LowIron	11	319
LowIron	12	321
.	.	.
.	.	.
.	.	.
None	26	238
None	27	259
None	28	243

This dataset could be analyzed using the One-Way Analysis of Variance procedure. The four groups would be compared by entering *Weight* as the Response Variable and *Food* as the Factor Variable.



## Output Excerpt – One-Way ANOVA Procedure

### Analysis of Variance Table and F-Test

Model Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Reject Equal Means? ( $\alpha = 0.05$ )	Power ( $\alpha = 0.05$ )
Between (Food)	3	64812.39	21604.13	20.6832	0.00000	Yes	1.00000
Within (Error)	24	25068.57	1044.524				
Adjusted Total	27	89880.96					
Total	28						

The equivalence of means is rejected (F-Ratio = 20.68, Prob Level = 0.00000) at the 0.05 alpha level.

## Setup

To run this example, complete the following steps:

### 1 Open Bean example dataset

- From the File menu of the NCSS Data window, select **Open Example Data**.
- Select **Bean** and click **OK**.

### 2 Specify the Mixed Models – General procedure options

- Find and open the **Mixed Models – General** procedure using the menus or the Procedure Navigator.
- The settings for this example are listed below and are stored in the **Example 3a** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

#### Variables Tab

Response Variable .....**Weight**  
 Subject Variable.....**Plot**  
 Repeated (Time) Variable.....**<Empty>**  
 Factor (Categorical) Variables .....**Food**  
 Fixed Effects Model .....**Food**

#### Comparisons Tab

Comparison .....**All Pairs**

### 3 Run the procedure

- Click the **Run** button to perform the calculations and generate the output.

## Mixed Models Output

### Repeated Component Parameter Estimates (R Matrix)

Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	1044.5238	Diagonal (Variance)

### Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Food	20.6832	3	24.0	0.000001

These F-Values test Type-III (adjusted last) hypotheses.

### Individual Comparison Hypothesis Test Results by Covariate Values

Comparison/ Covariate(s)	Comparison Mean Difference	F-Value	Num DF	Denom DF	Raw Prob Level	Bonferroni Prob Level
<b>Food</b>						
Food		20.6832	3	24.0	0.000001	
Food: HighIron - LowIron	59.2857	11.7774	1	24.0	0.002180	0.013080 [6]
Food: HighIron - None	121.7143	49.6401	1	24.0	0.000000	0.000002 [6]
Food: HighIron - Salicyl	110.0000	40.5448	1	24.0	0.000001	0.000008 [6]
Food: LowIron - None	62.4286	13.0592	1	24.0	0.001389	0.008335 [6]
Food: LowIron - Salicyl	50.7143	8.6181	1	24.0	0.007227	0.043363 [6]
Food: None - Salicyl	-11.7143	0.4598	1	24.0	0.504197	1.000000 [6]

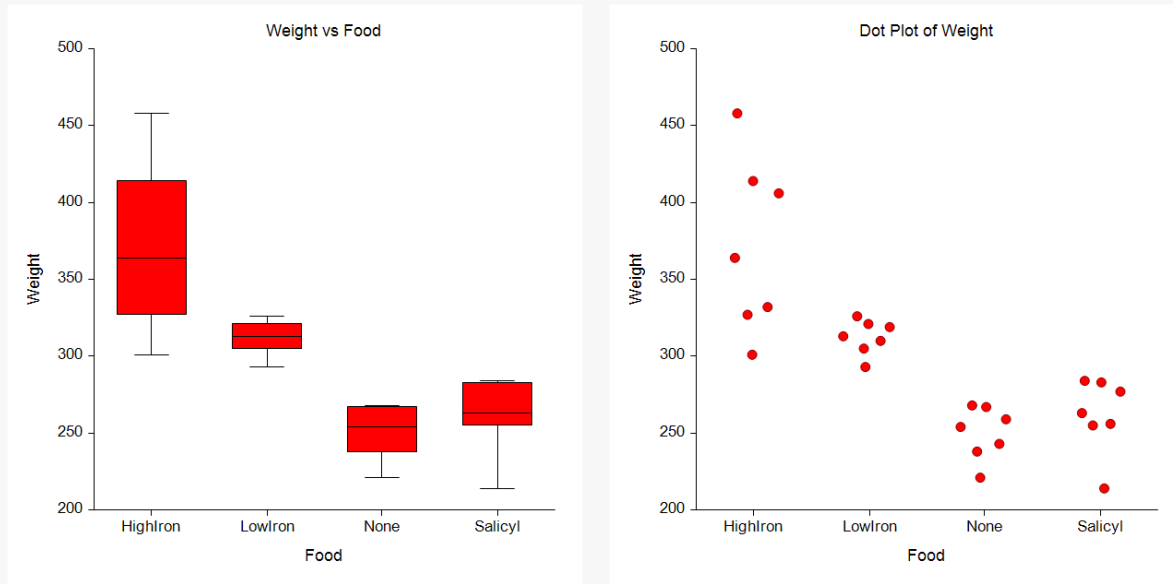
These F-Values test Type-III (adjusted last) hypotheses.

The overall F-test (F-Value = 20.68, Prob Level = 0.0000) comparing the means indicates there is strong evidence for differences among means. The individual comparison tests, with appropriate Bonferroni adjustments for multiple testing, indicate there are differences in means among all levels except between None and Salicylic acid.

The overall F-test (F-Value = 20.68, Prob Level = 0.0000) is identical to the one that results from the One-Way ANOVA procedure. If equal variances can reasonably assumed, the One-Way ANOVA procedure gives more detailed information than this procedure and should be used instead. However, for the case of unequal variances among groups, there is no way to use the One-Way Analysis of Variance procedure to analyze this dataset, except possibly with a transformation. In this example, the issue of unequal variances is important since the test for equal variance is rejected (see below).

Mixed Models - General

The following output is generated from the One-Way Analysis of Variance and Dot Plots procedures.



**Tests of the Equality of Group Variances Assumption**

Test Name	Test Value	Prob Level	Reject Equal Variances? ( $\alpha = 0.20$ )
Brown-Forsythe (Data - Medians)	6.2834	0.00267	Yes
Levene (Data - Means)	8.1436	0.00065	Yes
Conover (Ranks of Deviations)	12.5029	0.00584	Yes
Bartlett (Likelihood Ratio)	16.1344	0.00106	Yes

It appears HighIron group has a much larger variance than the other groups.

Comparing the levels of Food assuming different variance within each group using the Mixed Models procedure is shown in the next example.

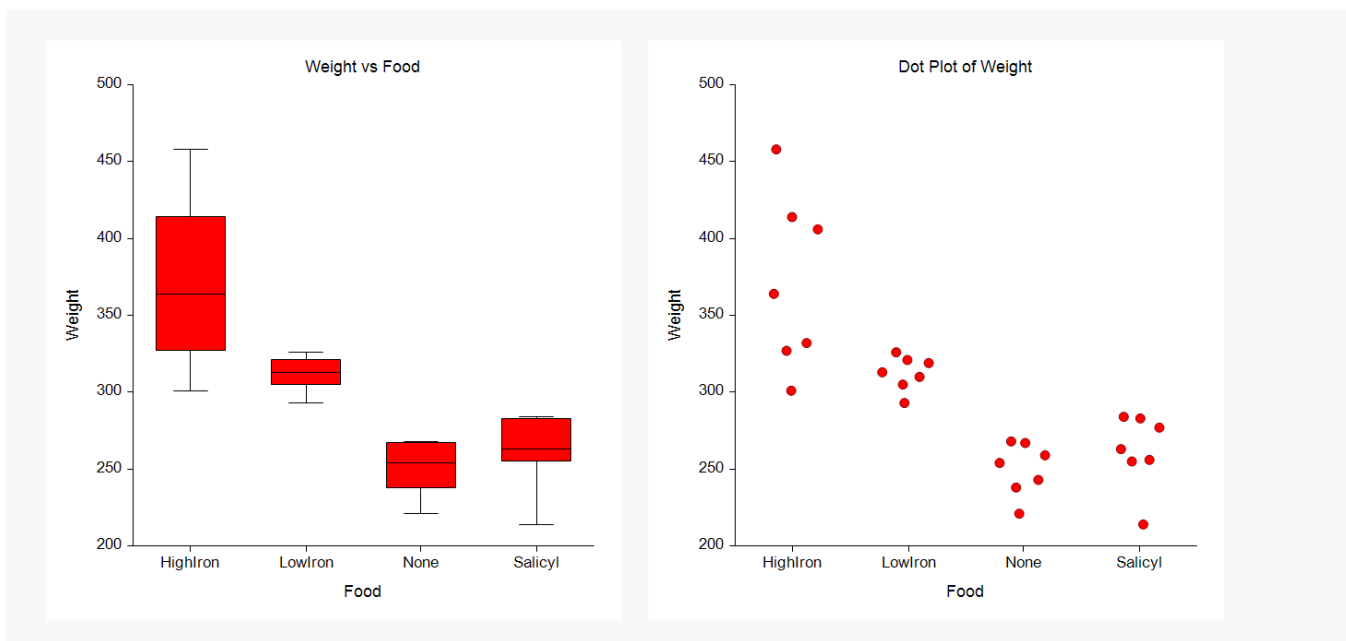
## Example 3b – One-Way ANOVA Design Assuming Unequal Variance (One Between-Subject Factor, No Within-Subject Factors, No Covariates)

In this example, four plant food mixtures (salicylic acid, low iron, high iron, and no food) are compared in their ability to promote growth in beans. Twenty-eight plots are used in the experiment. The response is the weight of the beans harvested from the plot. This example differs from the previous example in that the assumption of equal variances between groups is removed.

### Bean Dataset

Food	Plot	Weight
Salicyl	1	256
Salicyl	2	284
Salicyl	3	255
.	.	.
.	.	.
.	.	.
None	26	238
None	27	259
None	28	243

In this example, there is no way to use the One-Way Analysis of Variance procedure to analyze this dataset if unequal variances are assumed. The issue of unequal variances is important since an equal variance test is rejected (See the plots and equal variance test of the Bean data below). The following output is generated from the One-Way Analysis of Variance and Dot Plots procedures.



## Mixed Models - General

**Tests of the Equality of Group Variances Assumption**

Test Name	Test Value	Prob Level	Reject Equal Variances? ( $\alpha = 0.20$ )
Brown-Forsythe (Data - Medians)	6.2834	0.00267	Yes
Levene (Data - Means)	8.1436	0.00065	Yes
Conover (Ranks of Deviations)	12.5029	0.00584	Yes
Bartlett (Likelihood Ratio)	16.1344	0.00106	Yes

It appears HighIron group has a much larger variance than the other groups.

## Setup

To run this example, complete the following steps:

### 1 Open Bean example dataset

- From the File menu of the NCSS Data window, select **Open Example Data**.
- Select **Bean** and click **OK**.

### 2 Specify the Mixed Models - General procedure options

- Find and open the **Mixed Models - General** procedure using the menus or the Procedure Navigator.
- The settings for this example are listed below and are stored in the **Example 3b** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

#### Variables Tab

Response Variable ..... **Weight**  
 Subject Variable..... **Plot**  
 Repeated (Time) Variable..... **<Empty>**  
 Factor (Categorical) Variables ..... **Food**  
 Fixed Effects Model ..... **Food**  
 Repeated (Time) Covariance Pattern Groups ..... **Food**

#### Comparisons Tab

Comparison ..... **All Pairs**

### 3 Run the procedure

- Click the **Run** button to perform the calculations and generate the output.

## Mixed Models Output

### Repeated Component Parameter Estimates (R Matrix)

Component Number	Parameter Number	Group Number	Estimated Value	Parameter Type
1	1	1	3174.2381	Diagonal (Variance)
1	1	2	123.2857	Diagonal (Variance)
1	1	3	290.6667	Diagonal (Variance)
1	1	4	589.9048	Diagonal (Variance)

### Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Food	27.1319	3	10.8	0.000024

These F-Values test Type-III (adjusted last) hypotheses.

### Individual Comparison Hypothesis Test Results by Covariate Values

Comparison/ Covariate(s)	Comparison Mean Difference	F-Value	Num DF	Denom DF	Raw Prob Level	Bonferroni Prob Level
<b>Food</b>						
Food		27.1319	3	10.8	0.000024	
Food: HighIron - LowIron	59.2857	7.4612	1	6.5	0.031651	0.189909 [6]
Food: HighIron - None	121.7143	29.9288	1	7.1	0.000896	0.005376 [6]
Food: HighIron - Salicyl	110.0000	22.5018	1	8.2	0.001382	0.008292 [6]
Food: LowIron - None	62.4286	65.9044	1	10.3	0.000009	0.000051 [6]
Food: LowIron - Salicyl	50.7143	25.2437	1	8.4	0.000880	0.005283 [6]
Food: None - Salicyl	-11.7143	1.0909	1	10.8	0.319185	1.000000 [6]

These F-Values test Type-III (adjusted last) hypotheses.

The overall F-test comparing the means indicates there is strong evidence for differences among means. The individual comparison tests indicate there are differences in means among all levels except between None and Salicylic acid.

The Repeated Component Parameter Estimates section shows a different residual variance estimate for each of the four groups. The first variance estimate (3174.2381), corresponding to the high iron food mixture, is much larger than the others.

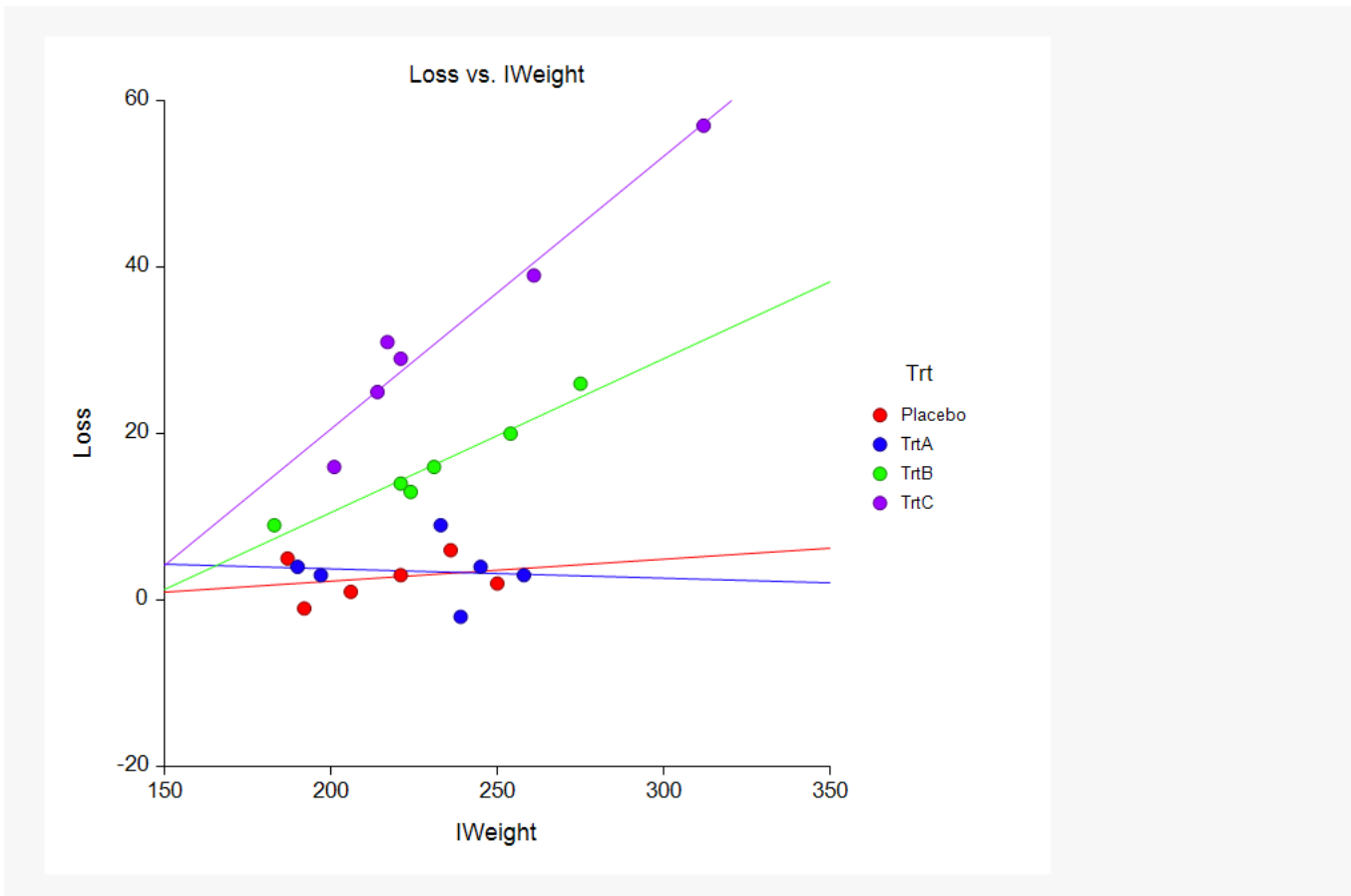
## Example 4 – ANCOVA Design (One Between-Subject Factor, No Within-Subject Factors, One Covariate)

In this example, three weight loss treatments (and a placebo) are compared. Twenty-four patients are randomly assigned to the three treatments and the placebo. Weight loss is the response. The weight of each participant before treatment is measured as a covariate. The researchers wish to compare the levels of Treatment at low (190 lbs.), medium (230 lbs.), and high (270 lbs.) values of initial weight.

### Weight Loss Dataset

Trt	Patient	IWeight	Loss
TrtA	1	197	3
TrtA	2	245	4
TrtA	3	233	9
TrtA	4	239	-2
TrtA	5	258	3
TrtA	6	190	4
TrtB	7	221	14
TrtB	8	231	16
TrtB	9	224	13
TrtB	10	183	9
TrtB	11	275	26
TrtB	12	254	20
.	.	.	.
.	.	.	.
.	.	.	.
Placebo	19	187	5
Placebo	20	192	-1
Placebo	21	250	2
Placebo	22	236	6
Placebo	23	221	3
Placebo	24	206	1

A scatter plot of the data is shown below.



This analysis could be run using the Multiple Regression procedure by entering Loss as the Dependent Variable, IWeight as a Numeric Independent Variable and Trt as a Categorical Independent Variable. The Default Contrast Type is set to Standard Set. The Custom Model is Trt|IWeight.

## Output Excerpt – Multiple Regression Procedure

### Analysis of Variance Detail

Source	DF	R <sup>2</sup> Lost If Term(s) Removed	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (5%)
Intercept	1		4592.667	4592.667			
Model	7	0.9682	4695.169	670.7384	69.613	0.0000	1.0000
IWeight	1	0.0005	2.207768	2.207768	0.229	0.6386	0.0736
Trt	3	0.0331	160.7248	53.57495	5.560	0.0083	0.8710
IWeight*Trt	3	0.0822	398.6192	132.8731	13.790	0.0001	0.9990
Error	16	0.0318	154.1646	9.635287			
Total(Adjusted)	23		4849.333	210.8406			

The significant IWeight\*Trt interaction (F-Ratio = 13.790, Prob Level = 0.0001) indicates there are differences among the slopes of the treatment groups. These results will be compared to those of the Mixed Models procedure in the output and discussion that follows.



## Setup

To run this example, complete the following steps:

### 1 Open Weight Loss example dataset

- From the File menu of the NCSS Data window, select **Open Example Data**.
- Select **Weight Loss** and click **OK**.

### 2 Specify the Mixed Models - General procedure options

- Find and open the **Mixed Models - General** procedure using the menus or the Procedure Navigator.
- The settings for this example are listed below and are stored in the **Example 4** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

Variables Tab

Response Variable .....**Loss**  
 Subject Variable.....**Patient**  
 Repeated (Time) Variable.....**<Empty>**  
 Factor (Categorical) Variables .....**Trt**  
 Covariate (Continuous) Variables.....**IWeight**

### 3 Run the procedure

- Click the **Run** button to perform the calculations and generate the output.

## Mixed Models Output

### Repeated Component Parameter Estimates (R Matrix)

Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	9.6353	Diagonal (Variance)

### Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Trt	5.5603	3	16.0	0.008274
IWeight	31.7737	1	16.0	0.000037
Trt*IWeight	13.7903	3	16.0	0.000106

These F-Values test Type-III (adjusted last) hypotheses.

Mixed Models - General

**Individual Comparison Hypothesis Test Results by Covariate Values**

Covariates: IWeight = 227.8333

Comparison/ Covariate(s)	Comparison Mean Difference	F-Value	Num DF	Denom DF	Raw Prob Level	Bonferroni Prob Level
<b>Trt</b>						
Trt		89.7772	3	16.0	0.000000	
Trt: Placebo - TrtA	-0.4899	0.0649	1	16.0	0.802207	1.000000 [3]
Trt: Placebo - TrtB	-12.6863	43.2391	1	16.0	0.000006	0.000019 [3]
Trt: Placebo - TrtC	-26.6099	186.0704	1	16.0	0.000000	0.000000 [3]

These F-Values test Type-III (adjusted last) hypotheses.

**Least Squares (Adjusted) Means by Covariate Values**

Covariates: IWeight = 227.8333

Name	Mean	Standard Error of Mean	95.0% Lower Conf. Limit for Mean	95.0% Upper Conf. Limit for Mean	DF
<b>Intercept</b>					
Intercept	12.9470	0.6635	11.5405	14.3535	16.0
<b>Trt</b>					
Placebo	3.0005	1.4465	-0.0659	6.0669	16.0
TrtA	3.4904	1.2679	0.8025	6.1783	16.0
TrtB	15.6868	1.2767	12.9804	18.3932	16.0
TrtC	29.6104	1.3089	26.8357	32.3852	16.0

The Term-by-Term Hypothesis Test Results are identical to those given in the Multiple Regression procedure output.

The Prob Level for the interaction Trt\*IWeight confirms what is seen in the scatter plot: the slopes differ for the different treatments. Two important sections of the output that are available in the Mixed Models procedure that are not available in the Multiple Regression procedure are mean comparisons and least squares (adjusted) means at specific values of the covariates.

The Individual Comparison Hypothesis Tests of the preceding output, however, are not very useful. They compare the placebo to each of the treatments at the mean of the covariate (IWeight = 227.83). To better understand the nature of the interaction, it is useful to compare the placebo to the three treatments at various values of the covariate. Some caution should be exercised with the number of values that are chosen because the probability levels (P-values) are adjusted according to the number of comparisons that are tested. Generally a low, medium, and high value of the covariate should suffice.

The researchers wished to compare the levels of Treatment at low (190 lbs.), medium (230 lbs.), and high (270 lbs.) values of initial weight. Such values should be chosen prior to collecting the data or at least before looking at the results. Comparisons and means at these values of the covariate are output by selecting the Covariate tab, selecting IWeight under Covariate Variable, and entering 190 230 270 under Compute Means at these Values. The relevant output is shown in the section that follows.

## Mixed Models - General

## Individual Comparison Hypothesis Test Results by Covariate Values

Covariates: IWeight = 190.0000

Comparison/ Covariate(s)	Comparison Mean Difference	F-Value	Num DF	Denom DF	Raw Prob Level	Bonferroni Prob Level
<b>Trt</b>						
Trt		11.2974	3	16.0	0.000316	0.000947 [3]
Trt: Placebo - TrtA	-1.9360	0.4287	1	16.0	0.521912	1.000000 [9]
Trt: Placebo - TrtB	-6.7081	5.2586	1	16.0	0.035722	0.321495 [9]
Trt: Placebo - TrtC	-15.2204	29.9618	1	16.0	0.000051	0.000459 [9]

These F-Values test Type-III (adjusted last) hypotheses.

Covariates: IWeight = 230.0000

Comparison/ Covariate(s)	Comparison Mean Difference	F-Value	Num DF	Denom DF	Raw Prob Level	Bonferroni Prob Level
<b>Trt</b>						
Trt		94.2700	3	16.0	0.000000	0.000000 [3]
Trt: Placebo - TrtA	-0.4071	0.0424	1	16.0	0.839378	1.000000 [9]
Trt: Placebo - TrtB	-13.0287	43.6944	1	16.0	0.000006	0.000054 [9]
Trt: Placebo - TrtC	-27.2622	188.3200	1	16.0	0.000000	0.000000 [9]

These F-Values test Type-III (adjusted last) hypotheses.

Covariates: IWeight = 270.0000

Comparison/ Covariate(s)	Comparison Mean Difference	F-Value	Num DF	Denom DF	Raw Prob Level	Bonferroni Prob Level
<b>Trt</b>						
Trt		79.3289	3	16.0	0.000000	0.000000 [3]
Trt: Placebo - TrtA	1.1218	0.0728	1	16.0	0.790744	1.000000 [9]
Trt: Placebo - TrtB	-19.3492	24.2421	1	16.0	0.000153	0.001374 [9]
Trt: Placebo - TrtC	-39.3040	112.9663	1	16.0	0.000000	0.000000 [9]

These F-Values test Type-III (adjusted last) hypotheses.

Mixed Models - General

**Least Squares (Adjusted) Means by Covariate Values**

**Covariates: IWeight = 190.0000**

Name	Mean	Standard Error of Mean	95.0% Lower Conf. Limit for Mean	95.0% Upper Conf. Limit for Mean	DF
<b>Intercept</b>					
Intercept	7.9562	1.0555	5.7188	10.1937	16.0
<b>Trt</b>					
Placebo	1.9901	1.8983	-2.0342	6.0144	16.0
TrtA	3.9261	2.2668	-0.8794	8.7315	16.0
TrtB	8.6982	2.2256	3.9801	13.4164	16.0
TrtC	17.2105	2.0318	12.9033	21.5177	16.0

**Covariates: IWeight = 230.0000**

Name	Mean	Standard Error of Mean	95.0% Lower Conf. Limit for Mean	95.0% Upper Conf. Limit for Mean	DF
<b>Intercept</b>					
Intercept	13.2329	0.6701	11.8123	14.6535	16.0
<b>Trt</b>					
Placebo	3.0584	1.5085	-0.1395	6.2562	16.0
TrtA	3.4655	1.2764	0.7597	6.1712	16.0
TrtB	16.0870	1.2686	13.3977	18.7764	16.0
TrtC	30.3206	1.2927	27.5801	33.0610	16.0

**Covariates: IWeight = 270.0000**

Name	Mean	Standard Error of Mean	95.0% Lower Conf. Limit for Mean	95.0% Upper Conf. Limit for Mean	DF
<b>Intercept</b>					
Intercept	18.5095	1.2396	15.8816	21.1374	16.0
<b>Trt</b>					
Placebo	4.1266	3.3028	-2.8750	11.1283	16.0
TrtA	3.0048	2.5253	-2.3485	8.3582	16.0
TrtB	23.4759	2.1297	18.9612	27.9906	16.0
TrtC	43.4306	1.6633	39.9047	46.9566	16.0

Examination of the individual comparison hypothesis tests shows that the mean difference from the placebo for those with a higher initial weight is greater than the mean difference for those with a lower initial weight, with the exception of Treatment A, for which there is no significant improvement in weight loss over the placebo.

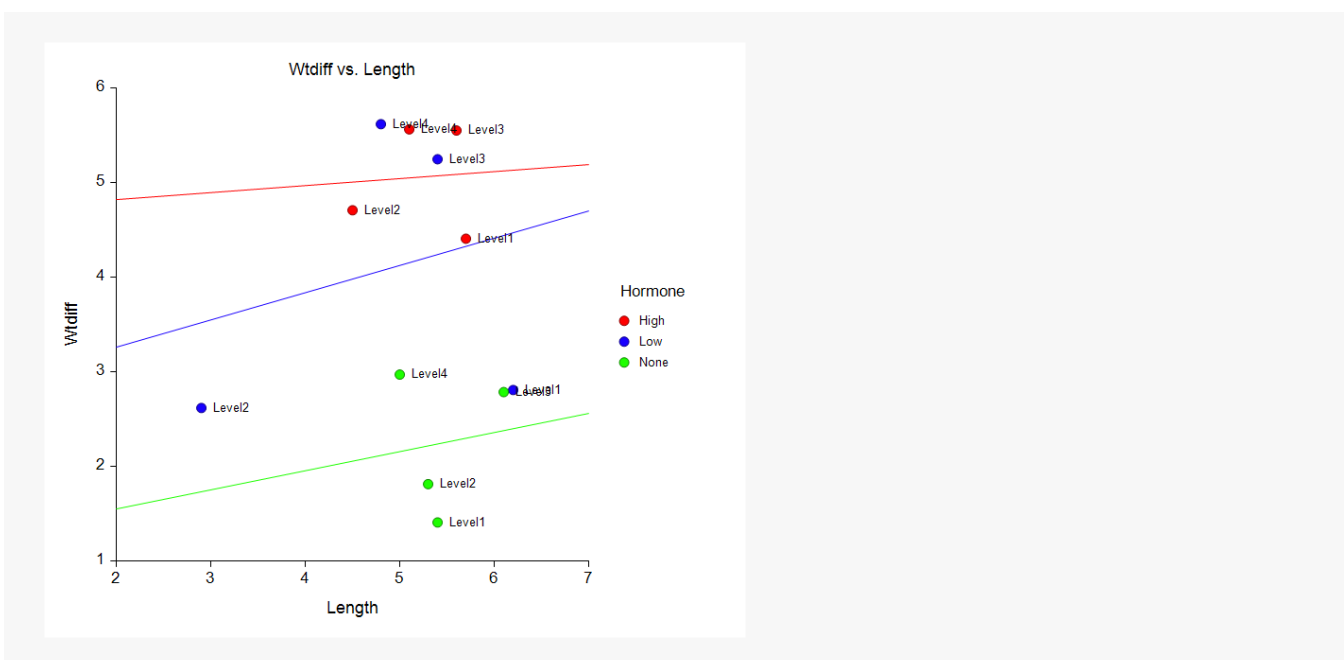
## Example 5 – Factorial Design (Two Between-Subject Factors, No Within-Subject Factors, One Covariate)

In a factorial design, more than one fixed factor is analyzed in a single experiment. One variable contains the response and two or more other variables identify the groups. In this example, a study is conducted to determine the effect of a growth hormone on trout growth at fish hatcheries. Twelve fish are compared in the study. Each of the 12 fish receives a different combination of hormone dose (none, low, or high) and amount of fish food (Level 1, Level 2, Level 3, or Level 4). The response is increase in weight after 3 weeks in the tank. The length of each fish prior to treatment is measured as a covariate.

### Fish Dataset

Fish	Food	Hormone	Length	Wtdiff
1	Level1	None	5.4	1.408
2	Level1	Low	6.2	2.808
3	Level1	High	5.7	4.407
4	Level2	None	5.3	1.813
5	Level2	Low	2.9	2.618
6	Level2	High	4.5	4.708
7	Level3	None	6.1	2.786
8	Level3	Low	5.4	5.247
9	Level3	High	5.6	5.551
10	Level4	None	5.0	2.971
11	Level4	Low	4.8	5.618
12	Level4	High	5.1	5.563

A scatter plot of the data is shown below.



This analysis could be run using the Multiple Regression procedure by entering Wtdiff as the Dependent Variable, Length as a Numeric Independent Variable, and Food and Hormone as Categorical Independent

Variables. The Default Contrast Type is set to Standard Set. The Custom Model is Food+Hormone+Length+Hormone\*Length.

## Output Excerpt – Multiple Regression Procedure

### Analysis of Variance Detail

Source	DF	R <sup>2</sup> Lost If Term(s) Removed	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (5%)
Intercept	1		172.5057	172.5057			
Model	8	0.9426	25.00806	3.126008	6.155	0.0813	0.5013
Length	1	0.0000	4.306755E-05	4.306755E-05	0.000	0.9932	0.0500
Food	3	0.3088	8.192474	2.730825	5.377	0.1003	0.4209
Hormone	2	0.0042	0.1111072	0.05555359	0.109	0.8998	0.0571
Length*Hormone	2	0.0028	0.07335056	0.03667528	0.072	0.9319	0.0547
Error	3	0.0574	1.523545	0.5078484			
Total(Adjusted)	11		26.53161	2.411964			

None of the terms of the model are significant in this example. However, the model can be refined. These results will be compared to those of the Mixed Models procedure in the output and discussion that follows.

## Setup

To run this example, complete the following steps:

### 1 Open Fish example dataset

- From the File menu of the NCSS Data window, select **Open Example Data**.
- Select **Fish** and click **OK**.

### 2 Specify the Mixed Models – General procedure options

- Find and open the **Mixed Models – General** procedure using the menus or the Procedure Navigator.
- The settings for this example are listed below and are stored in the **Example 5** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

#### Variables Tab

Response Variable ..... **WTDiff**  
 Subject Variable..... **Fish**  
 Repeated (Time) Variable..... **<Empty>**  
 Factor (Categorical) Variables ..... **Food-Hormone**  
 Covariate (Continuous) Variables ..... **Length**  
 Fixed Effects Model ..... **Food Hormone Length Hormone\*Length**

#### Comparisons Tab

Comparison ..... **All Pairs**

### 3 Run the procedure

- Click the **Run** button to perform the calculations and generate the output.

## Mixed Models Output

### Repeated Component Parameter Estimates (R Matrix)

Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	0.5078	Diagonal (Variance)

### Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Food	5.3772	3	3.0	0.100307
Hormone	0.1094	2	3.0	0.899798
Length	0.0106	1	3.0	0.924527
Hormone*Length	0.0722	2	3.0	0.931898

These F-Values test Type-III (adjusted last) hypotheses.

None of the factors are significant in this example. The model should be refined before examining the remainder of the output. It is evident that the length of the fish prior to treatment has little effect on the response, or at least with this small sample size the effect of length is not detectable. Removing the two non-significant terms associated with the length covariate and re-running the analysis gives the output that follows.

### Repeated Component Parameter Estimates (R Matrix)

Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	0.3076	Diagonal (Variance)

### Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Food	9.0896	3	6.0	0.011930
Hormone	26.4908	2	6.0	0.001053

These F-Values test Type-III (adjusted last) hypotheses.

## Mixed Models - General

## Individual Comparison Hypothesis Test Results by Covariate Values

Comparison/ Covariate(s)	Comparison Mean Difference	F-Value	Num DF	Denom DF	Raw Prob Level	Bonferroni Prob Level
<b>Food</b>						
Food		9.0896	3	6.0	0.011930	
Food: Level1 - Level2	-0.1720	0.1443	1	6.0	0.717163	1.000000 [6]
Food: Level1 - Level3	-1.6537	13.3347	1	6.0	0.010685	0.064110 [6]
Food: Level1 - Level4	-1.8430	16.5630	1	6.0	0.006578	0.039465 [6]
Food: Level2 - Level3	-1.4817	10.7051	1	6.0	0.016995	0.101968 [6]
Food: Level2 - Level4	-1.6710	13.6157	1	6.0	0.010209	0.061256 [6]
Food: Level3 - Level4	-0.1893	0.1748	1	6.0	0.690431	1.000000 [6]
<b>Hormone</b>						
Hormone		26.4908	2	6.0	0.001053	
Hormone: High - Low	0.9845	6.3017	1	6.0	0.045883	0.137650 [3]
Hormone: High - None	2.8128	51.4387	1	6.0	0.000371	0.001113 [3]
Hormone: Low - None	1.8283	21.7319	1	6.0	0.003461	0.010383 [3]

These F-Values test Type-III (adjusted last) hypotheses.

With the removal of the covariate terms, the results now show strong evidence of differences between levels of Food (F-Value = 9.09, Prob Level = 0.0119) and Hormone dose (F-Value = 26.49, Prob Level = 0.0011). Individual comparisons indicate evidence that the Level 1 mean is different from the Level 4 mean (Bonferroni Prob Level = 0.0395). The High and the Low levels of Hormone are significantly different (Bonferroni Prob Levels = 0.0011 and 0.0104, respectively) from the level None.

Similar results can be obtained using the General Linear Models procedure. However, the General Linear Models procedure would not allow the user to model different variances among groups, if this were desired.



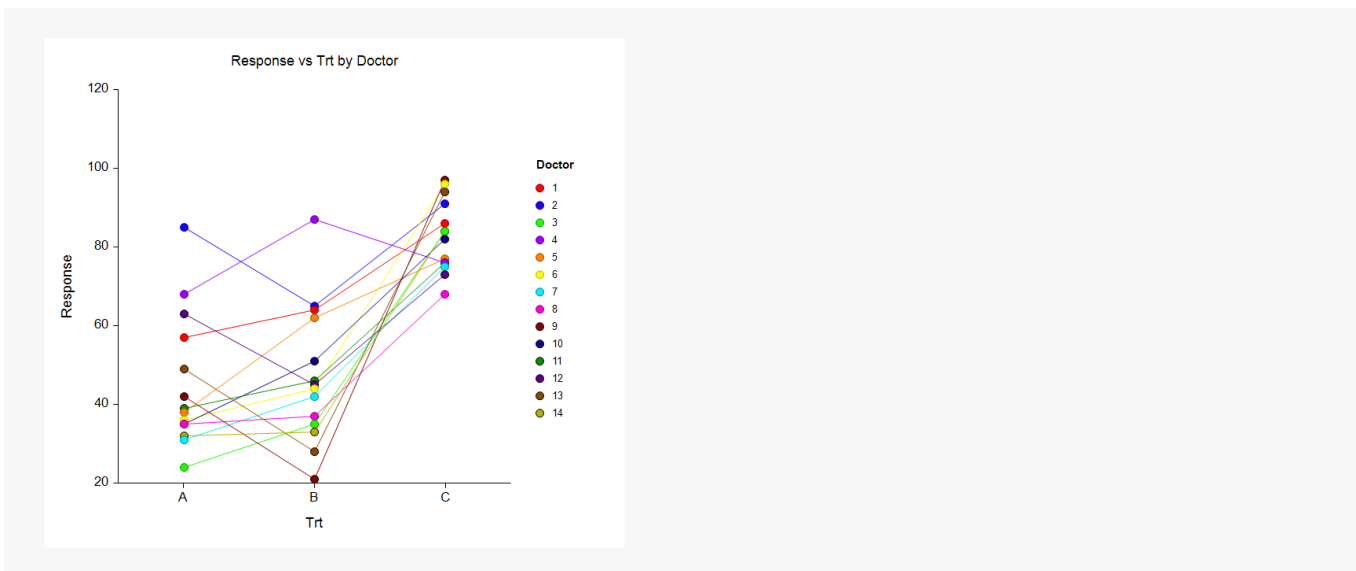
## Example 6 – Randomized Complete Block Design (No Between-Subject Factors, One Within-Subject Factor, No Covariates)

In a study to compare 3 treatments, three patients from each of 14 doctors are randomly assigned to each of the three treatments. A single response is measured for each patient following treatment. The result is a randomized complete block design with 14 blocks (doctors). The goal is to determine whether there are any differences among the three treatments. The data are contained in the RCBD data set.

### RCBD Dataset

Doctor	Patient	Trt	Response
1	1	A	57
1	2	B	64
1	3	C	86
2	4	A	85
2	5	B	65
.	.	.	.
.	.	.	.
.	.	.	.
13	38	B	28
13	39	C	94
14	40	A	32
14	41	B	33
14	42	C	84

A plot showing the 3 patients for each doctor is shown below.



Because there are no covariates, this analysis could be run using the Repeated Measures Analysis of Variance procedure by entering Response as the Response Variable, Doctor as the Subject Variable, and Trt as Within Factor 1. The Model Specification is Full model except subject interactions combined with error.

## Output Excerpt – Repeated Measures Analysis of Variance Procedure

**Analysis of Variance Table**

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Doctor	13	4232.119	325.5476	1.83	0.091201	
B: Trt	2	12507.19	6253.595	35.23	0.000000*	1.000000
S	26	4614.81	177.4927			
Total (Adjusted)	41	21354.12				
Total	42					

\* Term significant at alpha = 0.05

The difference in Treatment levels is highly significant (F-Ratio = 35.23, Prob Level = 0.000000). These results will be compared to those of the Mixed Models procedure in the output and discussion that follows.

### Setup

To run this example, complete the following steps:

#### 1 Open RCBD example dataset

- From the File menu of the NCSS Data window, select **Open Example Data**.
- Select **RCBD** and click **OK**.

#### 2 Specify the Mixed Models – General procedure options

- Find and open the **Mixed Models – General** procedure using the menus or the Procedure Navigator.
- The settings for this example are listed below and are stored in the **Example 6** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

Variables Tab

Response Variable ..... **Response**  
 Subject Variable..... **Doctor**  
 Repeated (Time) Variable..... **<Empty>**  
 Factor (Categorical) Variables ..... **Trt**  
 Fixed Effects Model ..... **Trt**  
 Random Model (Subject Terms Only)..... **Doctor**

Comparisons Tab

Comparison ..... **All Pairs**

#### 3 Run the procedure

- Click the **Run** button to perform the calculations and generate the output.

## Mixed Models Output

### Random Component Parameter Estimates (G Matrix)

Component Number	Parameter Number	Estimated Value	Model Term
1	1	49.3516	Doctor

### Repeated Component Parameter Estimates (R Matrix)

Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	177.4927	Diagonal (Variance)

### Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Trt	35.2330	2	26.0	0.000000

These F-Values test Type-III (adjusted last) hypotheses.

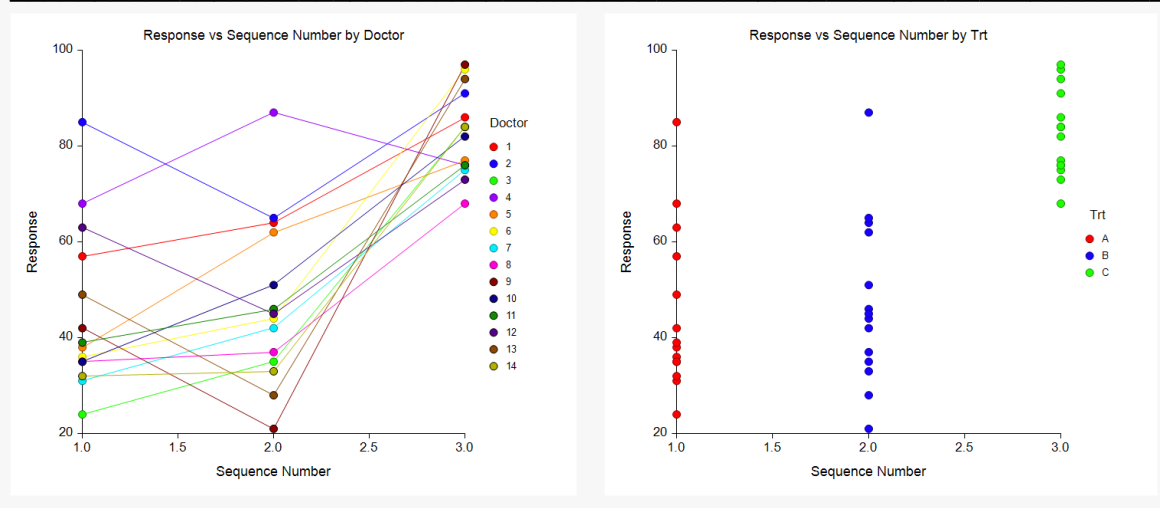
### Individual Comparison Hypothesis Test Results by Covariate Values

Comparison/ Covariate(s)	Comparison Mean Difference	F-Value	Num DF	Denom DF	Raw Prob Level	Bonferroni Prob Level
Trt						
Trt		35.2330	2	26.0	0.000000	
Trt: A - B	-1.8571	0.1360	1	26.0	0.715255	1.000000 [3]
Trt: A - C	-37.5000	55.4600	1	26.0	0.000000	0.000000 [3]
Trt: B - C	-35.6429	50.1029	1	26.0	0.000000	0.000000 [3]

These F-Values test Type-III (adjusted last) hypotheses.

Mixed Models - General

Subject Plots



The results of this test match those of the Repeated Measures ANOVA procedure (F-Value = 35.23, Prob Level = 0.0000). All the reports indicate that the mean response for Treatment C is much higher than A and B (the Bonferroni Prob Levels for A vs. C and B vs. C are both extremely small).

The second subject plot seems to indicate that the variation within responses of Treatment C is considerably smaller than the variation within Treatments A and B. This can be accounted for by entering Trt for Groups in the Repeated Variance Pattern on the Variables tab.

Random Component Parameter Estimates (G Matrix)

Component Number	Parameter Number	Estimated Value	Model Term
1	1	8.7505	Doctor

Repeated Component Parameter Estimates (R Matrix)

Component Number	Parameter Number	Group Number	Estimated Value	Parameter Type
1	1	1	276.0158	Diagonal (Variance)
1	1	2	297.4464	Diagonal (Variance)
1	1	3	76.3315	Diagonal (Variance)

Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Trt	41.4257	2	17.1	0.000000

These F-Values test Type-III (adjusted last) hypotheses.

The conclusions do not change when the unequal variance is accounted for, but the estimated variances are indeed quite different across treatments. The estimated variances for Treatments A and B are 276.0158 and 297.4464, respectively, while the estimated variance for Treatment C is only 76.3315. These tests based on unequal variance assumptions are more accurate than those where equal variances were assumed.

## Example 7 – Complex Split-Plot Design (One Between-Subject Factor, Two Within-Subject Factors, Two Covariates)

In a standard split-plot design, plots are randomized to a between-plot treatment and are also sub-divided, with each sub-division receiving a different within-plot treatment. This example involves a more complex split-design with an additional within-plot factor and two covariates.

In a study to compare the effectiveness of 3 tutoring methods, 84 students (42 male, 42 female) are randomly assigned to 14 tutors (7 graduates, 7 undergraduates) in groups of 6 (3 male, 3 female). Each tutor uses a different tutoring method for each student according to the scheme below. A pre-exam is administered to each student before the semester of tutoring begins. IQ is also obtained for each student. The response is the score on an exam taken at the end of the semester.

### Tutor Dataset

Educ	Tutor	Student	Method	Gender	Preexam	IQ	Exam
Undergr	1	1	A	M	45	117	70
Undergr	1	2	A	F	35	113	84
Undergr	1	3	B	M	68	94	95
Undergr	1	4	B	F	47	103	77
Undergr	1	5	C	M	25	100	78
Undergr	1	6	C	F	24	95	88
Undergr	2	7	A	M	16	96	80
Undergr	2	8	A	F	38	99	75
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
Grad	14	79	A	M	27	109	77
Grad	14	80	A	F	36	104	81
Grad	14	81	B	M	24	79	84
Grad	14	82	B	F	27	99	72
Grad	14	83	C	M	33	93	75
Grad	14	84	C	F	39	109	63

The only procedure that can be used to incorporate all the variables of this analysis in a single model is the Mixed Models procedure.

## Setup

To run this example, complete the following steps:

### 1 Open Tutor example dataset

- From the File menu of the NCSS Data window, select **Open Example Data**.
- Select **Tutor** and click **OK**.

### 2 Specify the Mixed Models – General procedure options

- Find and open the **Mixed Models – General** procedure using the menus or the Procedure Navigator.
- The settings for this example are listed below and are stored in the **Example 7** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

Variables Tab

---

Response Variable ..... **Exam**  
 Subject Variable..... **Tutor**  
 Repeated (Time) Variable..... **<Empty>**  
 Factor (Categorical) Variables ..... **Educ,Method-Gender**  
 Covariate (Continuous) Variables ..... **Preexam-IQ**  
 Fixed Effects Model ..... **Educ Method Gender Preexam IQ**  
 Random Model (Subject Terms Only)..... **Tutor**

### 3 Run the procedure

- Click the **Run** button to perform the calculations and generate the output.

## Mixed Models Output

#### Random Component Parameter Estimates (G Matrix)

Component Number	Parameter Number	Estimated Value	Model Term
1	1	0.8100	Tutor

#### Repeated Component Parameter Estimates (R Matrix)

Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	79.4941	Diagonal (Variance)

#### Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Educ	0.6770	1	14.3	0.424099
Method	0.9964	2	65.8	0.374712
Gender	0.2135	1	65.4	0.645537
Preexam	0.0525	1	76.1	0.819412
IQ	1.0666	1	77.0	0.304948

These F-Values test Type-III (adjusted last) hypotheses.

There is no statistical evidence of differences among the levels of Education, Method, or Gender (all Prob Levels > 0.05).

## Example 8 – Cross-Over Design (No Between-Subject Factors, Two Within-Subject Factors, One Covariate)

In a basic two-level cross-over design, each subject receives both treatments, but (approximately) half receive the two treatments in the opposite order. In this example, researchers are comparing two drugs for their effect on heart rate in rats. Each rat is given both drugs, with a short washout period between drug administrations, but the order of the drugs is reversed in half of the rats. An initial heart rate (IHR) measurement is taken immediately before administration of each of the drugs.

### Cross Dataset

Rat	Period	Trtcross	IHR	HR
1	1	Drug A	389	357
1	2	Drug B	383	381
2	1	Drug B	372	409
2	2	Drug A	390	385
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
20	1	Drug B	408	403
20	2	Drug A	391	371

## Setup

To run this example, complete the following steps:

### 1 Open Cross example dataset

- From the File menu of the NCSS Data window, select **Open Example Data**.
- Select **Cross** and click **OK**.

### 2 Specify the Mixed Models – General procedure options

- Find and open the **Mixed Models – General** procedure using the menus or the Procedure Navigator.
- The settings for this example are listed below and are stored in the **Example 8** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

#### Variables Tab

```

Response Variable .....HR
Subject Variable.....Rat
Repeated (Time) Variable.....<Empty>
Factor (Categorical) Variables .....Period-Trtcross
Covariate (Continuous) Variables .....IHR
Fixed Effects Model .....Trtcross Period IHR
Random Model (Subject Terms Only).....Rat
    
```

### 3 Run the procedure

- Click the **Run** button to perform the calculations and generate the output.

## Mixed Models Output

### Random Component Parameter Estimates (G Matrix)

Component Number	Parameter Number	Estimated Value	Model Term
1	1	6.9232	Rat

### Repeated Component Parameter Estimates (R Matrix)

Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	189.6101	Diagonal (Variance)

### Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Trtcross	3.9878	1	20.6	0.059200
Period	0.4899	1	17.5	0.493163
IHR	2.0013	1	35.6	0.165844

These F-Values test Type-III (adjusted last) hypotheses.

The F-test for Trtcross is nearly significant (F-value = 3.9978, Prob Level = 0.0592) at the 0.05 level. There appears to be no period effect (F-value = 0.49, Prob Level = 0.4932) nor relationship between the initial heart rate (F-value = 2.0013, Prob Level = 0.1658) and the response heart rate.

The advantages of using mixed models in cross-over designs are usually more pronounced when there is missing data. Missing values often occur in cross-over designs when subjects fail to appear for the second treatment. Another advantage of mixed models in cross-over designs over conventional analyses occurs when there are three or more treatments involved. In such cases, the cross-over design may be considered a repeated measures design, and specific covariate patterns can be used to model the similarity in repeated measurements. That is, measurements that are taken closer together may be expected to vary more similarly, while measurements at distant periods may not. The Mixed Models procedure provides greater flexibility in modeling options for such situations.



## Example 9 – Random Coefficients Model (One Between-Subject Factor, No Within-Subject Factors, One Covariate, Unequal Time Points, Missing Data)

Researchers would like to determine the effect of a new hair loss treatment. Eighteen men are randomly divided into two groups. One group receives the placebo (shampoo without treatment), the other group receives the hair loss treatment (shampoo with treatment). The participants are asked to shampoo daily and return to the lab after every two months for one year. At each visit, participants are given a hair re-growth score. As is sometimes the case with human subjects, the return visits were not as scheduled. Some participants returned before or after the scheduled two month period, while some others dropped out of the study.

### Hair Dataset

Treatment	Individual	Time	Regrowth
Placebo	1	2	14
Placebo	1	4	5
Placebo	1	7	3
Placebo	1	9	7
Placebo	1	12	8
Placebo	2	2	6
Placebo	2	4	3
Placebo	3	2	5
Placebo	3	5	2
Placebo	3	10	4
Placebo	4	2	7
Placebo	4	4	9
.	.	.	.
.	.	.	.
.	.	.	.
Trt	17	6	7
Trt	17	12	3
Trt	18	2	7
Trt	18	4	10
Trt	18	6	14
Trt	18	8	17

### Setup

To run this example, complete the following steps:

#### 1 Open Hair example dataset

- From the File menu of the NCSS Data window, select **Open Example Data**.
- Select **Hair** and click **OK**.

## 2 Specify the Mixed Models – General procedure options

- Find and open the **Mixed Models – General** procedure using the menus or the Procedure Navigator.
- The settings for this example are listed below and are stored in the **Example 9** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

Variables Tab

---

Response Variable .....**Regrowth**  
 Subject Variable.....**Individual**  
 Repeated (Time) Variable.....**Time**  
 Factor (Categorical) Variables .....**Treatment**  
 Covariate (Continuous) Variables.....**Time**  
 Fixed Effects Model .....**Treatment Time Treatment\*Time**  
 Random Model (Subject Terms Only).....**Individual Individual\*Time**  
 Covariances.....**Checked**

## 3 Run the procedure

- Click the **Run** button to perform the calculations and generate the output.

## Mixed Models Output

### Random Component Parameter Estimates (G Matrix)

Component Number	Parameter Number	Estimated Value	Model Term
1	1	16.6319	Individual
1	2	1.8015	Individual*Time
1	3	-4.2599	Individual, Individual*Time

### Repeated Component Parameter Estimates (R Matrix)

Component Number	Parameter Number	Estimated Value	Parameter Type
1	1	5.9013	Diagonal (Variance)

### Term-by-Term Hypothesis Test Results

Model Term	F-Value	Num DF	Denom DF	Prob Level
Treatment	0.2042	1	13.6	0.658459
Time	7.7449	1	12.3	0.016219
Treatment*Time	9.4960	1	12.3	0.009259

These F-Values test Type-III (adjusted last) hypotheses.

Mixed Models - General

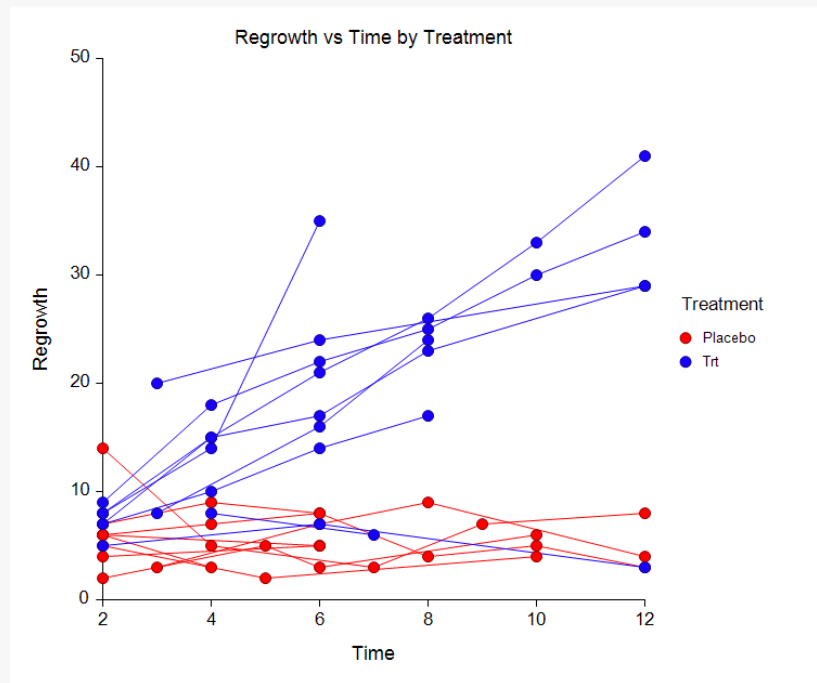
**Individual Comparison Hypothesis Test Results by Covariate Values**

Covariates: Time = 6.0000

Comparison/ Covariate(s)	Comparison Mean Difference	F-Value	Num DF	Denom DF	Raw Prob Level	Bonferroni Prob Level
<b>Treatment</b>						
Treatment		18.7211	1	15.1	0.000593	
Treatment: Placebo - Trt	-11.8425	18.7211	1	15.1	0.000593	0.000593 [1]

These F-Values test Type-III (adjusted last) hypotheses.

**Subject Plots**



The significant Treatment\*Time interaction (F-Value = 9.50, Prob Level = 0.0093) indicates that the differences between the Treatment and the Placebo are different at different times. If comparisons are made at times 2, 7, and 12, the results are

**Individual Comparison Hypothesis Test Results by Fixed Effects**

Comparison/ Covariate(s)	Comparison Mean Difference	F-Value	Num DF	Denom DF	Raw Prob Level	Bonferroni Prob Level
<b>Treatment: Placebo - Trt</b>						
Time = 2.0000	-3.2053	3.9286	1	14.4	0.066901	0.200704 [3]
Time = 7.0000	-14.0017	17.5590	1	14.5	0.000843	0.002528 [3]
Time = 12.0000	-24.7981	13.8925	1	13.2	0.002479	0.007438 [3]

These F-Values test Type-III (adjusted last) hypotheses.

There is strong evidence that the difference in means increases as time increases.