## Unit 8.

**Introduction to Repeated Measurements Analysis**

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</table>
1. Introduction and Examples

This unit is a very basic introduction to concepts and methods for the description and analysis of repeated measurements data.

- Introduced are graphical and numerical summaries, simple analysis methods that focus on univariate summaries (e.g., change over time, slope, area under the curve, etc), univariate and multivariate analysis of variance and, an introduction to the normal theory mixed model.

- Only the continuous outcome setting is considered and normality is assumed.

- Not discussed are repeated measurements of discrete outcomes.

- A more rigorous treatment of this topic is beyond the scope of this course.

Repeated measures data are unlike the kinds of data we have been studying so far.

In the settings considered so far, the available data was comprised of one observation per subject only and we assumed mutual independence of subject responses.

* Unit 2 – Multivariable Regression

The available data are $Y_1 \ldots Y_n$, mutually independent, one observation per subject. For each $i$, $Y_i$ is assumed to be a realization of a $N(\mu_i, \sigma_{y|x}^2)$.

$$
\mu_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \ldots + \beta_p X_{pi}
$$

$$
\sigma_{y|x}^2 = \text{constant}
$$

We explored the estimation and interpretation of the normal theory multivariable linear model $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_p X_p$ + error

In one example, we studied investigated the relationship between a random variable $Y =$ length of hospital stay and two fixed predictors

$X_1 =$ age, continuous is fixed
$X_2 =$ history of prior vertebral fracture (1=yes, 0=no)
* Unit 5– Logistic Regression

The available data are Y₁ … Yₙ, again one observation per subject, mutually independent. Here, each Yᵢ is the realization of a Bernoulli distribution with parameter \( \piᵢ \) and

\[
\text{logit} [\piᵢ] = \beta₀ + \beta₁X₁ + \beta₂X₂ + \beta₃X₃ + \beta₄X₄ + \beta₅X₅ + \beta₆X₆
\]

Recall: \( \text{logit}[\pi] = \ln \left[ \frac{\pi}{1-\pi} \right] \)

As an example, we explored the relationship between the outcome event \( Y = \) spontaneous abortion and the fixed predictors

- \( X₁ = \) History of prior spontaneous abortion
- \( X₂ = \) Cigarette Smoking
- \( X₃ = \) History of thyroid condition

* Unit 6– Introduction to Survival Analysis

The available data are (X₁,C₁) … (Xₙ, Cₙ), one observation per subject, mutually independent where X = period of observation and C = 0/1 an indicator variable that recorded event occurrence (1=yes, 0=no). Of interest was the (possibly incompletely observed) “time to event” random variable T. We considered only the setting of right censoring. Thus,

\( Xᵢ = Tᵢ \) when \( Cᵢ = 1 \) (Event occurrence known. \( T = X \))
\( < Tᵢ \) when \( Cᵢ = 0 \) (Event occurrence NOT known. \( T > X \))

The probability distribution of T was not considered in our introduction. We considered only model free or semi-parametric approaches to data analysis and one focus was on the hazard function. (the instantaneous probability of event at time “t”, given survival up to time “t”).

Recall \( hₜ(t⁺) = \lim_{\Delta t \to 0} \left( \frac{\text{Prob}[t⁺ ≤ T < t⁺ + \Delta t | T ≥ t⁺]}{\Delta t} \right) \)
Our introduction to the Cox proportional hazard model allowed us to explore the relationship of time to event to fixed predictor variables, which we represented as $Z_1, Z_2, \ldots Z_p$, so as to avoid confusion with $X = \text{period of observation}$.

$$h(t; Z_1, \ldots Z_p) = h_0(t) \exp[\beta_1 Z_1 + \cdots + \beta_p Z_p],$$

The example we utilized investigated the prognostic significance of blood pressure (low versus normal) for duration of survival following a heart attack.

Repeated measures data contain multiple and interrelated observations for each individual.

Here are three examples.

* **Example 1– Intervention Study of Flaxseed (Sturgeon, S)**

Sturgeon et al conducted a one sample intervention study to investigate the potential health benefits of ingestion of ground flaxseed on selected hormones that are hypothesized to be associated with increased risk of breast cancer. $N=48$ healthy postmenopausal women were enrolled.

The repeated measurements data consist of repeated measurements of each of serum testosterone, estrone, estradiol and sex hormone binding globulin on three occasions: (1) Baseline, (2) 6 weeks; and (3) 12 weeks.
* Example 2– Effect of Diet on Glucose Tolerance in Mice (in progress)

Glucose tolerance levels over time, in relationship to diet, were measured in six groups of mice, according to the diet they received: (1) low fat control; (2) high fat control; (3) intervention low dose; (4) intervention medium dose; (5) intervention high dose; and (6) sibutramine. There were n=14 mice in each group, for a total sample size of N=84.

The repeated measurements consisted of measurements of oral glucose tolerance test values (OGTT) on five occasions: (1) pre-treatment; (2) time 0; (3) time 30 minutes; (4) time 60 minutes; and (5) time 120 minutes.

Here is a plot of the group means over time.
* Example 3– Growth Curve Analysis


This example is often used in introductions to repeated measurements analysis. It’s a nice example to work with.

This was a study of two groups of children, 11 girls and 16 boys. Repeated measurements of a dental measure were made for each child on four occasions of age, in years: 8, 10, 12, and 14.

Here are the data, reproduced directly from the Potthoff and Roy (1964) article.

<table>
<thead>
<tr>
<th>Girls</th>
<th>Age in years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>21</td>
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<tr>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>20.5</td>
</tr>
<tr>
<td>4</td>
<td>23.5</td>
</tr>
<tr>
<td>5</td>
<td>21.5</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>21.5</td>
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<td>8</td>
<td>23</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>16.5</td>
</tr>
<tr>
<td>11</td>
<td>24.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Boys</th>
<th>Age in years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>21.5</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>25.5</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
</tr>
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<td>6</td>
<td>24.5</td>
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<tr>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>9</td>
<td>23</td>
</tr>
<tr>
<td>10</td>
<td>27.5</td>
</tr>
<tr>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>12</td>
<td>21.5</td>
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<tr>
<td>13</td>
<td>17</td>
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<tr>
<td>14</td>
<td>22.5</td>
</tr>
<tr>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td>16</td>
<td>22</td>
</tr>
</tbody>
</table>

Mean: 21.18 22.23 23.09 24.09
Mean: 22.87 23.81 25.72 27.47
Here are the individual growth profiles for the children, separately for boys and girls, using the software package MS Excel; later we’ll see how to obtain these plots using SAS and STATA.
Goals of Repeated Measurements Analyses –

Associated with repeated measures data are a variety of research questions. Some pertain to means (location), others to variances and covariances (variability).

Location -

- What is the nature or form of the profile over time (or over the occasions of measurement, more generally) of the repeated measurements?

- Are there group differences in the profiles over time (or over the occasions of measurement, more generally)?

- Do there exist covariates/predictors of the repeated measures profiles? What is the nature and significance of these predictors?

Variability -

- What is the nature and magnitude of the covariation/auto-correlation among repeated measurements within subject? (serial correlation)

- What is the nature of and magnitude of the heterogeneity of repeated measurements profiles between subjects? (random effects)

- Do there exist group differences in the within or between subjects patterns of variability?
2. Basic Concepts

Repeated measurements data arise in many fields of study so that there are a variety of terminologies.

**Definition -**

Our definition of repeated measurements is measurements of the same characteristic on the same individual unit on more than one occasion (Crowder and Hand, p. 1)

- **Longitudinal** data are repeated measurements over time.

- The term **time series** is used generally to refer to a repeated measurements data setting where the number of repeated measurements over time is large compared to the number of subjects.

- **Clustered data** is another kind of repeated measurements data situation. In this setting, the cluster is the unit of measurement and the data consist of repeated measurements of the cluster. (eg. Children within a school).

- **Familial data** is also a type of repeated measurements data setting. Here, the pedigree is the unit of measurement and the data consist of measurements of multiple individuals within each pedigree.

- **Dose-response data** are sometimes (but not always, depending on the design) repeated measurements data. One focus of interest is the estimation of a dose-response relationship. (e.g. drug development studies). In this setting, the subject is the unit of measurement and the data consist of repeated measurements of each subject, each at all of the doses of interest.
Where possible, we will use the simplest notation possible and limit our need for vectors and matrices.

Notation -

- **Subjects** will be identified with “i” and range \( i = 1 \ldots N \)

- **Occasions of repeated measurements** will be identified with “t” and range \( t = 1 \ldots T \).  
  
  *Note – In more advanced settings, it is possible for the number of repeated measurements to differ from one subject to the next. We will not be considering that situation here.*

- The **outcome variable** will be represented using \( Y \). Thus, the profile of \( T \) repeated measurements for one subject “i” is \( (Y_{i1}, Y_{i2}, \ldots, Y_{iT}) \)

- **Predictor variables** will be represented using \( X \). These will be indexed using “j” and will range \( j = 1 \ldots P \).

Data Layout -

There are two kinds of data layouts, **univariate (long)** and **multivariate (wide)**. Consider an example where the number of repeated measurements is \( T = 4 \) and the number of covariates \( X \) is \( p = 1 \):

- **Layout is Univariate/Long**

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Occasion</th>
<th>( Y )</th>
<th>( X )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-7</td>
<td>3.5</td>
<td>45</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>3.7</td>
<td>45</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>3.9</td>
<td>45</td>
</tr>
<tr>
<td>1</td>
<td>60</td>
<td>3.0</td>
<td>45</td>
</tr>
</tbody>
</table>

... Rows omitted ...

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Occasion</th>
<th>( Y )</th>
<th>( X )</th>
</tr>
</thead>
<tbody>
<tr>
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<td>-7</td>
<td>4.2</td>
<td>51</td>
</tr>
<tr>
<td>N</td>
<td>0</td>
<td>4.7</td>
<td>51</td>
</tr>
<tr>
<td>N</td>
<td>30</td>
<td>5.0</td>
<td>51</td>
</tr>
<tr>
<td>N</td>
<td>60</td>
<td>4.7</td>
<td>51</td>
</tr>
</tbody>
</table>
• Typically, this is used for univariate repeated measurements analyses

• **Layout is Multivariate/Wide**

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Y_{11}</th>
<th>Y_{12}</th>
<th>...</th>
<th>Y_{1T}</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.5</td>
<td>3.7</td>
<td>3.0</td>
<td>45</td>
<td></td>
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<tr>
<td>N</td>
<td>4.2</td>
<td>4.7</td>
<td>4.7</td>
<td>51</td>
<td></td>
</tr>
</tbody>
</table>

... Rows omitted ...

• Use this for multivariate repeated measurements analysis approaches

• Notice the absence of recorded information on the actual occasions of measurements in the multivariate/wide layout. Thus, this layout is meaningful only when the occasions of measurement are the same for all subjects.

The Potthoff and Roy data have been posted for you, in both formats, in an excel workbook.  
[http://www-unix.oit.umass.edu/~biep640w/data%20sets/potthoff_roy.xls](http://www-unix.oit.umass.edu/~biep640w/data%20sets/potthoff_roy.xls)
Central to the concept of repeated measurements is that the repeated measurements within each subject are correlated.

- Each subject “i” contributes to the data set a profile of T measurements Y. Using the notation developed here, we have for each subject “i”, ranging i = 1 …. N, a profile (vector) of T repeated measurements:

  \[Y_{i1} = 1^{\text{st}} \text{ measurement for the “i”-th subject}\]
  \[Y_{i2} = 2^{\text{nd}} \text{ measurement for the “i”-th subject}\]
  \[\ldots \text{ etc } \ldots\]
  \[Y_{iT} = T^{\text{th}} \text{ measurement for the “i”-th subject}\]

- Consider the variability among the repeated measurements of one individual subject “i”. Within this one subject, each of the T repeated measurements has a variance. Also, for each subject “i”, any pair of the T repeated measurements will have a covariance. These variances and covariances are represented in what is called a variance-covariance structure (“matrix”):

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>\ldots \ldots</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cov(Y₁,Y₁)</td>
<td>Cov(Y₁,Y₂)</td>
<td>\ldots \ldots</td>
<td>Cov(Y₁,Yₜ)</td>
</tr>
<tr>
<td>2</td>
<td>Cov(Y₂,Y₁)</td>
<td>Cov(Y₂,Y₂)</td>
<td>\ldots \ldots</td>
<td>Cov(Y₂,Yₜ)</td>
</tr>
<tr>
<td>\ldots</td>
<td>\ldots</td>
<td>\ldots</td>
<td>\ldots</td>
<td>\ldots</td>
</tr>
<tr>
<td>T</td>
<td>Cov(Yₜ,Y₁)</td>
<td>Cov(Yₜ,Y₂)</td>
<td>\ldots \ldots</td>
<td>Cov(Yₜ,Yₜ)</td>
</tr>
</tbody>
</table>

- Note on the diagonal entries of this matrix – The covariance of a random variable with itself is the same thing as the variance. Thus, Cov(Y₁, Y₁) = Var(Y₁), Cov(Y₂, Y₂) = Var(Y₂), \ldots, Cov(Yₜ, Yₜ) = Var(Yₜ).
Consider next a closer look at the covariance among any two repeated measurements on the same individual subject “i”.

- Index the two occasions of measurement using subscripts “t” and “t*”

\[ \text{Cov}(Y_t, Y_{t*}) = \text{Covariance}(Y_t, Y_{t*}) = \mathbb{E} \left[ (Y_t - \mathbb{E}[Y_t])(Y_{t*} - \mathbb{E}[Y_{t*}]) \right] = \sigma_{t,t*} \]

where

- \( \sigma_{t,t*} = \rho_{t,t*} \sigma_t \sigma_{t*} \)

and where

\[ \sigma_t = \sqrt{\sigma_{tt}} = \sqrt{\sigma_t^2} \]

- Correlation\( (Y_t, Y_{t*}) = \rho_{t,t*} \)

In general, \( \text{Covariance} (Y_t, Y_{t*}) = \sigma_{t,t*} \)

Let’s look at what this means, depending on \( t \) and \( t* \).

<table>
<thead>
<tr>
<th>( t ) and ( t* ) are two different occasions</th>
<th>( t ) and ( t* ) are the same occasion = ( t )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Cov} (Y_t, Y_{t*}) = \sigma_{t,t*} )</td>
<td>( \text{Cov} (Y_t, Y_t) = \sigma_{t,t} = \sigma_t^2 = \text{Var}(Y_t) )</td>
</tr>
<tr>
<td>( \text{Correlation} (Y_t, Y_{t*}) = \rho_{t,t*} )</td>
<td>( \text{Correlation} (Y_t, Y_t) = \rho_{t,t} = 1 )</td>
</tr>
<tr>
<td>( \text{Cov}(Y_t, Y_{t*}) = \sigma_{t,t*} = \rho_{t,t*} \sigma_t \sigma_{t*} )</td>
<td>( \text{Cov}(Y_t, Y_t) = \sigma_{t,t} = \rho_{t,t} \sigma_t \sigma_t = \sigma_t^2 )</td>
</tr>
</tbody>
</table>

Putting this all together, an individual variance-covariance structure has the following general definition

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>\ldots</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \sigma_{11} = \sigma_1^2 )</td>
<td>( \sigma_{12} = \rho_{12} \sigma_1 \sigma_2 )</td>
<td>( \ldots )</td>
</tr>
<tr>
<td>2</td>
<td>( \sigma_{21} = \rho_{21} \sigma_2 \sigma_1 )</td>
<td>( \sigma_{22} = \sigma_2^2 )</td>
<td>( \ldots )</td>
</tr>
<tr>
<td>\ldots</td>
<td>( \ldots )</td>
<td>( \ldots )</td>
<td>( \ldots )</td>
</tr>
<tr>
<td>T</td>
<td>( \sigma_{T1} = \rho_{T1} \sigma_T \sigma_1 )</td>
<td>( \sigma_{T2} = \rho_{T2} \sigma_T \sigma_2 )</td>
<td>( \ldots )</td>
</tr>
</tbody>
</table>
Notice the following.

1. Variances can be homogeneous or heterogeneous and
2. Covariances can be homogeneous or heterogeneous

Some specific selected variance-covariance structures have been given names. Here are some, in order, from completely general (unstructured) to most restrictive (compound symmetric).

- **Unstructured**

  **Variance-Covariance**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>...</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$\sigma_{11} = \sigma_1^2$</td>
<td>$\sigma_{12} = \rho_{12} \sigma_1 \sigma_2$</td>
<td>$\ldots$</td>
<td>$\sigma_{1T} = \rho_{1T} \sigma_1 \sigma_T$</td>
</tr>
<tr>
<td>2</td>
<td>$\sigma_{21} = \rho_{21} \sigma_2 \sigma_1$</td>
<td>$\sigma_{22} = \sigma_2^2$</td>
<td>$\ldots$</td>
<td>$\ldots$</td>
</tr>
<tr>
<td>...</td>
<td>$\ldots$</td>
<td>$\ldots$</td>
<td>$\ldots$</td>
<td>$\ldots$</td>
</tr>
<tr>
<td>T</td>
<td>$\sigma_{T1} = \rho_{T1} \sigma_T \sigma_1$</td>
<td>$\sigma_{T2} = \rho_{T2} \sigma_T \sigma_2$</td>
<td>$\sigma_{TT} = \sigma_T^2$</td>
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</tr>
</tbody>
</table>

  **Correlation Structure**

<table>
<thead>
<tr>
<th></th>
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<th>2</th>
<th>3</th>
<th>...</th>
<th>T-2</th>
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<th>T</th>
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<td>1</td>
<td>$\rho_{12}$</td>
<td>$\rho_{13}$</td>
<td>$\ldots$</td>
<td>$\rho_{1T}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>$\rho_{21}$</td>
<td>1</td>
<td>$\rho_{23}$</td>
<td>$\ldots$</td>
<td>$\rho_{2T}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>...</td>
<td>$\ldots$</td>
<td>$\ldots$</td>
<td>$\ldots$</td>
<td>$\ldots$</td>
<td>$\ldots$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>$\rho_{T1}$</td>
<td>$\rho_{T2}$</td>
<td>$\rho_{T3}$</td>
<td>$\ldots$</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- *This is completely general and therefore the most complex. It has the largest number of unknown parameters that require estimation.*

- *Often, it is not necessary to utilize this most complex structure.*
• First order ante-dependence

\[
\begin{array}{c|cccc}
\text{Var-Covariance} & 1 & 2 & \ldots & T \\
1 & \sigma_{11} = \sigma_1^2 & \sigma_{12} = \rho_1 \sigma_1 \sigma_2 & \ldots & \sigma_{1T} = \rho_1 \rho_2 \ldots \rho_{T-1} \sigma_1 \sigma_T \\
2 & \sigma_{12} = \rho_1 \sigma_1 \sigma_2 & \sigma_{22} = \sigma_2^2 & \ldots & \ldots \\
\vdots & \vdots & \vdots & \ddots & \vdots \\
T & \sigma_{1T} = \rho_1 \rho_2 \ldots \rho_{T-1} \sigma_1 \sigma_T & \sigma_{TT} = \sigma_T^2 & \ldots & \ldots \\
\end{array}
\]

This allows the variances to change with occasion of repeated measurement and is therefore general in this respect.

The correlation between pairs of repeated measurements in this structure is assumed to be the product of the correlations between adjacent times and is therefore restricted in this respect.

Estimation of this variance-covariance structure requires estimating \(2T-1\) parameters.

• Toeplitz

\[
\begin{array}{c|cccc}
\text{Var-Covariance} & 1 & 2 & \ldots & T \\
1 & \sigma_{11} = \sigma^2 & \sigma_{12} = \rho_1 \sigma^2 & \ldots & \sigma_{1T} = \rho_{T-1} \sigma^2 \\
2 & \sigma_{21} = \rho_1 \sigma^2 & \sigma_{22} = \sigma^2 & \ldots & \ldots \\
\vdots & \vdots & \vdots & \ddots & \vdots \\
T & \sigma_{T1} = \rho_{T-1} \sigma^2 & \sigma_{TT} = \sigma^2 & \ldots & \ldots \\
\end{array}
\]

In the Toeplitz model, the variance of outcome at each occasion of measurement is restricted to an assumed common value.

The correlation between pairs of repeated measurements that have the same lag ‘d” have a common correlation \(\rho_d\)

Estimation of this variance-covariance structure requires estimating \(T\) parameters.
• **First order autoregressive**  

**Variance-Covariance**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>............</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(\sigma_{11} = \sigma^2)</td>
<td>(\sigma_{12} = \rho \sigma^2)</td>
<td>(\ldots)</td>
<td>(\sigma_{1T} = \rho^{T-1}\sigma^2)</td>
</tr>
<tr>
<td>2</td>
<td>(\sigma_{21} = \rho \sigma^2)</td>
<td>(\sigma_{22} = \sigma^2)</td>
<td>(\ldots)</td>
<td>(\ldots)</td>
</tr>
<tr>
<td>...</td>
<td>(\ldots)</td>
<td>(\ldots)</td>
<td>(\ldots)</td>
<td>(\ldots)</td>
</tr>
<tr>
<td>T</td>
<td>(\sigma_{T1} = \rho^{T-1}\sigma^2)</td>
<td>(\ldots)</td>
<td>(\ldots)</td>
<td>(\sigma_{TT} = \sigma^2)</td>
</tr>
</tbody>
</table>

• Like the Toeplitz model, in the first order autoregressive model, the variance of outcome at each occasion of measurement is restricted to an assumed common value.

• In a first order autoregressive model, the correlation between any pair of repeated measurements that have lag 1 is \(\rho^1\), the correlation between any pair of repeated measurements that have lag 2 is \(\rho^2\) ... and the correlation between the pair of repeated measurements that have lag “T-1” is \(\rho^{T-1}\).

• Estimation of this variance-covariance structure requires estimating only 2 parameters.

• **Compound Symmetric (Exchangeable)**  

**Variance-Covariance**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>............</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(\sigma_{11} = \sigma^2)</td>
<td>(\sigma_{12} = \rho \sigma^2)</td>
<td>(\ldots)</td>
<td>(\sigma_{1T} = \rho \sigma^2)</td>
</tr>
<tr>
<td>2</td>
<td>(\sigma_{21} = \rho \sigma^2)</td>
<td>(\sigma_{22} = \sigma^2)</td>
<td>(\ldots)</td>
<td>(\ldots)</td>
</tr>
<tr>
<td>...</td>
<td>(\ldots)</td>
<td>(\ldots)</td>
<td>(\ldots)</td>
<td>(\ldots)</td>
</tr>
<tr>
<td>T</td>
<td>(\sigma_{T1} = \rho \sigma^2)</td>
<td>(\ldots)</td>
<td>(\ldots)</td>
<td>(\sigma_{TT} = \sigma^2)</td>
</tr>
</tbody>
</table>

• The variance of outcome at each occasion of measurement is restricted to an assumed common value.

• And, it is assumed that the correlation between any two repeated measurements is the same REGARDLESS of the lag.

• Estimation of this variance-covariance structure also requires estimating only 2 parameters.

*Note – This is the covariance structure that is assumed in a univariate repeated measurements analysis of variance and in a split model analysis of variance. More on this in Section 5.*
3. Description and Summarization

The complexity of repeated measurements data, with its many interrelationships, makes data description essential before doing any model estimation and testing.

- The goals of description should correspond to the goals of the analyses. Recall that some of these goals are listed on page 8.

- Summaries of location reveal means and changes in mean over the occasions of repeated measures. Graphical summaries, especially, give a feel for the functional form of the repeated measurements means over occasion.

- Summaries of variance-covariance provide clues to the variances of the repeated measures (are the variances constant or do they change over time?) and the covariances (is there an autocorrelation structure?)

It’s helpful to obtain both graphical and numerical descriptions.

- Some graphical summaries
  - Side-by-side box and whisker plots – While side-by-side box plots do not summarize the interrelationships among the repeated measures themselves, this plot is still useful. It provides a sense of equality of means and equality of variances.
  - Line plots or spaghetti plots - These give a feel for the raw data profiles and can reveal unusual individuals.
  - Mean response profiles, with or without associated SE or confidence bands. These give a feel for the functional form of the response profile. In settings where there are multiple groups, these also give a feel for group differences.
  - Matrix Scatterplot- This gives you a visual feel for the autocorrelation structure.
Some numerical summaries

- **Means, SD and sample size at each occasion of repeated measurement, separately by group as appropriate** – This is the numerical companion to the side by side box and whisker plot.

- **One way analysis of variance of group differences at baseline (or the 1st occasion of measurement)** – This is useful in studies of change over time. Here it is important to assess comparability of groups at baseline so that data on changes over time can be meaningfully interpreted in relationship to intervention or group.
a. Side by Side Box and Whisker Plots

Source: Potthoff and Roy (1964)

SAS

* Data are in long format;

* Sort data by both group (X) and time (t);
proc sort data=temp;
by x t;
run;

* Side by side box and whisker of outcome Y;
proc boxplot data=temp;
plot y*t;
by x;
title "Potthoff and Roy (1964)";
run;
quit;
**STATA**

.* Data are in long format.*
  . use "Z:\bigelow\teaching\web640\stata\roy_long.dta", clear

  . * Box and Whisker Plot for Girls Using Same Y-axis as for Boys*
  . drop if group==2
  . sort age
  . graph box growth, over(age) title(“Girls”) ti(“Age, years”) ylabel(10 “10” 15 “15” 20 “20” 25 “25” 30 “30” 35 “35”)

  . * Box and Whisker Plot for Boys Using Same Y-axis as for Girls*
  . clear
  . use "Z:\bigelow\teaching\web640\stata\roy_long.dta", clear
  . drop if group==1
  . sort age
  . graph box growth, over(age) title(“Boys”) ti(“Age, years”) ylabel(10 “10” 15 “15” 20 “20” 25 “25” 30 “30” 35 “35”)

• Variances at each age occasion appear to be the same within gender and across gender

• Dental growth over time is seen in both genders

• Slightly greater growth with age in boys is suggested.
b. Plots of Individual Profiles

*Source: Potthoff and Roy (1964)*

**SAS**

```sas
* Data are in long format;

* Girls;
symbol1 i=join v=none l=1 r=27;
symbol2 i=join v=none l=2 r=34;
proc gplot data=temp;
  where x=1;
  plot y*t=id/nolegend skipmiss; /*notice the use of id */
  title "Girls";
run;
quit;

* Boys;
symbol1 i=join v=none l=1 r=27;
symbol2 i=join v=none l=2 r=34;
proc gplot data=temp;
  where x=2;
  plot y*t=id/nolegend skipmiss;
  title "Boys";
run;
quit;
```

![Graphs of Girls and Boys growth data](image)
STATA

. *Data are in long format.
. use "Z:\bigelow\teaching\web640\stata\roy_long.dta", clear

. * the option c(l) is the letter l and tells STATA to connect points with a line
. * Line plots for Girls
. drop if group==2
. sort age
. xtline growth, t(age) i(id) overlay title("Girls") xlabel(8 “8” 10 “10” 12 “12” 14 “14”) ylabel(10 “10” 15 “15” 20 “20” 25 “25” 30 “30” 35 “35”) t1("Girls")

. * Line plots for Boys
. clear
. use "Z:\bigelow\teaching\web640\stata\roy_long.dta", clear
. drop if group==1
. sort age
. xtline growth, t(age) i(id) overlay title("Girls") xlabel(8 “8” 10 “10” 12 “12” 14 “14”) ylabel(10 “10” 15 “15” 20 “20” 25 “25” 30 “30” 35 “35”) t1("Girls")

• The profiles over time are a little more variable among the boys.
• Data for boys includes 1-3 profiles that are outlying
• These profiles may influence comparison of groups.
c. Plots of Mean Response Profiles

Source: Potthoff and Roy (1964)

SAS

* Data are in long format;

goptions reset=symbol;
 SYMBOL1 = stdm1j l=1;
 SYMBOL2 = stdm1j l=2;

proc gplot data=temp;
  plot y*t=x;
  title 'Mean Growth +/- 1 SE, by Group';
run;

Key –
- “stdm” instructs SAS to produce standard error bars.
- “std” would instruct SAS to produce standard deviation bars.
- The “1” in “stdm1” instructs SAS that length of bar should be ± 1 SE unit
- The “j” in “stdm1j” instructs SAS to connect/join the means.

Mean Growth +/- 1 SE, by Group

Graph showing mean growth over time with error bars.
**STATA**

`* Data are in long format.
. use "Z:\bigelow\teaching\web640\stata\roy_long.dta", clear

* Use collapse instruction to create a data set with mean, std, and n. Check.
. collapse (mean) growth (sd) sdgrowth=growth (count) n=growth, by(group age)
. list

<table>
<thead>
<tr>
<th>age</th>
<th>group</th>
<th>growth</th>
<th>sdgrowth</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>21.18182</td>
<td>2.124532</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>22.22727</td>
<td>1.902152</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>23.18182</td>
<td>2.442056</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>24.09091</td>
<td>2.437398</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>22.625</td>
<td>3.185906</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>23.8125</td>
<td>2.136001</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>25.71875</td>
<td>2.651847</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>27.4875</td>
<td>2.096624</td>
<td>16</td>
</tr>
</tbody>
</table>

* Tell STATA to produce +/- 1 se bars
. generate high=growth+1*sdgrowth/sqrt(n)
. generate low=growth-1*sdgrowth/sqrt(n)

* mean +/- SE plot for Girls
. graph twoway (connected growth age) (connected high age) (connected low age) if group==1, ylabel(10 15 20 25 30 35) xlabel(8 10 12 14) title("Girls") subtitle("Mean +/- 1 SE")

* mean +/- SE plot for Boys
. graph twoway (connected growth age) (connected high age) (connected low age) if group==2, ylabel(10 15 20 25 30 35) xlabel(8 10 12 14) title("Boys") subtitle("Mean +/- 1 SE")

2010
d. Scatterplot Matrix

Source: Potthoff and Roy (1964)

SAS
Obtaining a scatterplot matrix in SAS is a bit cumbersome depending on whether you want to write your own code in the program editor or from SAS/INSIGHT.

- If you are using the program editor, it is not difficult to locate and download a macro %SCATMAT. Lots of people have posted it in various ways. Do a google search to find one that you like.

SAS/INSIGHT
Use data in WIDE format.
The following assumes that the data are in roywide.sas7bdat

*Execute the following instruction using the correct path for the location of your data.
Libname class “z:\bigelow\teaching\web640\sas”;

Launch SAS/INSIGHT from the box in the upper left corner.
Choose library CLASS and member ROYWIDE
Click OPEN
Holding down the CTRL-key, highlight Y1 Y2 Y3 Y4

From the upper left corner, ANALYZE > SCATTERPLOT (Y X)

You should see …
STATA version 7
Obtaining a scatterplot matrix in STATA is very straightforward.
Use data in WIDE format.
The following assumes that the data are in *roy_wide.dta*

```
. use "Z:\bigelow\teaching\web640\stata\roy_wide.dta", clear
. graph matrix y1 y2 y3 y4
```

You should see
4. Simple Analyses of Derived Summaries

One approach to the analysis of repeated measures data is to compute for each subject profile of repeated measures a univariate summary. The univariate summary is then the object of analysis.

Beware that there are disadvantages, having to do with loss of information

- We do not learn about the shape of the repeated measures profiles
- Reduction of a multivariate random variable to a univariate summary risks obscuring important aspects of the data.
- Interpretation can be difficult.

However, there are some advantages to the analysis of a univariate summary

- Simple
- Quick
- Sometimes, these analyses are fully adequate to the question.

Some univariate summaries

1. Change Score = (Last repeated measure) - (First repeated measure)
2. Endpoint Analysis: Regression Analysis of last observation controlling for first observation
3. Average score, taken over all of the repeated measures
4. Linear trend = Least squares estimate of slope
5. Area under the curve

While these analyses are computationally familiar (you may have learned the techniques already), it’s helpful to clear on the underlying model and null hypotheses.
Univariate Summary #1.
Change Score = (Last repeated measure) - (First repeated measure)

\[ D_i = (Y_{iT} - Y_{i1}) = \text{(Last - First)}. \]

Model for 1 Group.
- \( D_i = \beta_0 + e_i \)
- Paired t-test is equivalent to test of \( H_0 : \beta_0 = 0 \)

Model for 2 Groups.
- Define \( X = 0 \) if control, 1 if treatment
- \( D_{ij} = \beta_0 + \beta_1 X_{ij} + e_{ij} \)
- \( H_0 : \beta_0 = 0 \) tests “Null: mean change is zero among controls”
- \( H_0 : \beta_1 = 0 \) tests “Null: mean change is same across groups”

Univariate Summary #2.
Endpoint Analysis of Covariance (ANCOVA) of Last Observation
The ANCOVA model includes a term for adjustment for pre/baseline (the first measure)

\[ Y_{iT} = \beta_0 + \beta_1 Y_{i1} + \beta_2 X_i + e_i \]

Model for 2 Groups.
- Define \( X = 0 \) if control, 1 if treatment
- \( H_0 : \beta_0 = 0 \) tests “Null: mean post (last repeated measure) is zero among controls with zero pre (first repeated measure)”
- \( H_0 : \beta_1 = 0 \) tests “Null: controlling for group, post is not related to pre”
- \( H_0 : \beta_2 = 0 \) tests “Null: controlling for pre, mean post is same across groups”

Summarizing …
<table>
<thead>
<tr>
<th>Change Score</th>
<th>Analysis of Covariance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is the average change over time the same across groups?</td>
<td>Is the average post the same across groups, among subpopulations where the pre is the same?</td>
</tr>
</tbody>
</table>
Univariate Summary #3.
Average Score, taken over all the repeated measures
This is equivalent to analyzing the univariate summary equal to the sum of the repeated measurements.

\[ S_i = \frac{(Y_{i1} + Y_{i2} + \ldots + Y_{iT})}{T} \]

Model for 2 Groups.
- Define X=0 if control, 1 if treatment
- \[ S_{ij} = \beta_0 + \beta_1 X_{ij} + e_{ij} \]
- \( H_0: \beta_0 = 0 \) is not usually of interest as it tests “Null: average of averages is zero among controls”
- \( H_0: \beta_1 = 0 \) is of interest but tests a limited null hypothesis: “average over time (or whatever the repeated measures axis is) is same across groups”

Univariate Summary #4.
Linear trend = Least Squares estimate of the slope.
This analysis focuses on the slope of the repeated measurements.
This could be the least squares slope using the actual repeated measurements or the ranks.
So as not to get confused by notation, lets call this univariate summary U.

\[ U_i = \text{least squares estimate of slope over the repeated measurements.} \]

Model for 2 Groups.
- Define X=0 if control, 1 if treatment
- \[ U_{ij} = \beta_0 + \beta_1 X_{ij} + e_{ij} \]
- \( H_0: \beta_0 = 0 \) tests “Null: mean slope of repeated measurements is zero among controls”
- \( H_0: \beta_1 = 0 \) tests “Null: mean slope of repeated measurements is same across groups”
Univariate Summary #5. 

Area under the Curve (AUC). 

Analysis focuses on the summary of the repeated measurements profile that is the area under the curve.

There are a variety of methods for calculating an area under the curve; they can be quite involved. The simplest is the following. For each individual, AUC is approximated using the “trapezoid” approach as follows.

\[
AUC = \left(\frac{1}{2}\right) \sum_{t=1}^{T-1} (t_{t+1} - t_t) [Y_t + Y_{t+1}]
\]

A feel for the “trapezoid” approach for AUC calculations is the following:

http://www.personal.psu.edu/jhm/f90/lectures/38i.html

We start with some x-y curve where y=f(x).

The integral (area under the curve) is approximated by the sum of the areas of n-1 trapezoids.

The area of one such trapezoid is:

\[
A_1 = 0.5 \left( y_{t+1} + y_t \right) (x_{t+1} - x_t)
\]

Model for 2 Groups.

- Define X=0 if control, 1 if treatment
- \(AUC_{ij} = \beta_0 + \beta_1X_{ij} + e_{ij}\)
- \(H_0: \beta_0 = 0\) is not of interest as it tests “Null: mean area under the curve is zero among controls”
- \(H_0: \beta_1 = 0\) tests “Null: mean area under the curve is same across groups”
Example of an Area Under the Curve Analysis

A 2 way factorial experiment examined the effect of compound at 2 levels (K1256 and K1397) and dose at 3 levels (0.01, .05, and .25) on levels of prolactin (ng/ml).

- The sample size is balanced, 6 per group, for a total of 36. Complete data that is analyzable is available for n=35. One animal is missing one measurement in the K1256 dose .01 group.

- The six study conditions (groups) are
  1 – Compound K1256 Dose .01
  2 – Compound K1256 Dose .05
  3 – Compound K1256 Dose .25
  4 – Compound K1397 Dose .01
  5 – Compound K1397 Dose .05
  6 – Compound K1397 Dose .25

  Data are repeated measurements of prolactin (ng/ml) on 5 occasions
  1 – baseline
  2 – 30 minutes
  3 – 60 minutes
  4 – 120 minutes
  5 – 240 minutes

- Consider two analysis questions

  1. For each compound, is there a dose effect on prolactin as measured by the area under the curve of the 5 repeated measurements?

  2. At each level of dose, is there an effect of compound on prolactin as measured by the area under the curve of the 5 repeated measurements?
Data Description for Area Under the Curve Values

Box and Whisker
Area Under Curve
Prolactin (ng/ml)

27997.9
4792.8

1=K1256_01  2=K1256_05  3=K1256_25  4=K1397_01  5=K1397_05  6=K1397_25
With Compound K1256, is there a **DOSE effect** on AREA UNDER CURVE (AUC)?

```plaintext
.* One way anova of DOSE effects, separately for each compound.
.sort compound
.by compound: oneway auc dose, tabulate obs means standard

<table>
<thead>
<tr>
<th></th>
<th>Summary of auc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>compound = 1</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>dose</td>
<td>Mean</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>.01</td>
<td>11271.69</td>
</tr>
<tr>
<td>.05</td>
<td>14711.15</td>
</tr>
<tr>
<td>.25</td>
<td>21807.65</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Total</td>
<td>16204.19</td>
</tr>
</tbody>
</table>

Analysis of Variance

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>323415390</td>
<td>2</td>
<td>161707695</td>
<td>5.96</td>
<td>0.0134</td>
</tr>
<tr>
<td>Within groups</td>
<td>379877291</td>
<td>14</td>
<td>27134092.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>703292681</td>
<td>16</td>
<td>43955792.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bartlett's test for equal variances: chi2(2) = 0.8367 Prob>chi2 = 0.658

Bartlett test for equality of variances (H₀: σ₁² = σ₂² = σ₃²) p-value = .067
One way analysis of variance test for equality of means (H₀: μ₁ = μ₂ = μ₃) p-value = .01

Discussion
The differences in variabilities seen in the box and whisker plot (boxes 1, 2, and 3) are of marginal significance (p=.067). One way parametric analysis of variance is statistically significant (p=.01). The trend observed is positive with dose.
With Compound K1397, is there a **DOSE effect** on **AREA UNDER CURVE (AUC)**?

<table>
<thead>
<tr>
<th></th>
<th>Summary of auc</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dose</td>
<td>Mean</td>
</tr>
<tr>
<td>-----</td>
<td>------</td>
<td>---------</td>
</tr>
<tr>
<td>.01</td>
<td>11527.975</td>
<td>3791.8209</td>
</tr>
<tr>
<td>.05</td>
<td>18682.85</td>
<td>1358.7584</td>
</tr>
<tr>
<td>.25</td>
<td>17024.475</td>
<td>938.82171</td>
</tr>
<tr>
<td>-----</td>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>Total</td>
<td>15745.1</td>
<td>3864.1298</td>
</tr>
</tbody>
</table>

**Analysis of Variance**

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>168307905</td>
<td>2</td>
<td>84153952.5</td>
<td>14.76</td>
<td>0.0003</td>
</tr>
<tr>
<td>Within groups</td>
<td>85527580.2</td>
<td>15</td>
<td>5701838.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>253835485</td>
<td>17</td>
<td>14931499.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bartlett's test for equal variances: chi2(2) = 9.5041  Prob>chi2 = 0.009

Bartlett test for equality of variances ($H_0: \sigma_1^2 = \sigma_2^2 = \sigma_3^2$) p-value = .009

One way analysis of variance test for equality of means ($H_0: \mu_1 = \mu_2 = \mu_3$) p-value = .0003

**Discussion**

The differences in variabilities seen in the box and whisker plot (boxes 4, 5, and 6) are highly statistically significant (p=.009), as is the one way parametric analysis of variance is statistically significant (p=.0003). From an initially relatively variable AUC response at dose .01, upward trend then levels off and AUC responses are less variable at doses .05 and .25.
At Dose = .01 mg/ml is there a **COMPOUND effect on (AUC)?**

```plaintext
.* One way anova of effect of COMPOUND, separately at each dosage.
. sort dose
. by dose: oneway auc compound, tabulate obs means standard

<table>
<thead>
<tr>
<th></th>
<th>Summary of auc</th>
</tr>
</thead>
<tbody>
<tr>
<td>compound</td>
<td>Mean    Std. Dev.</td>
</tr>
<tr>
<td>1</td>
<td>11271.69  6620.5349</td>
</tr>
<tr>
<td>2</td>
<td>11527.975 3791.8209</td>
</tr>
<tr>
<td>Total</td>
<td>11411.482 4973.8776</td>
</tr>
</tbody>
</table>

Analysis of Variance

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>179133.199</td>
<td>1</td>
<td>179133.199</td>
<td>0.01</td>
<td>0.9374</td>
</tr>
<tr>
<td>Within groups</td>
<td>247215455</td>
<td>9</td>
<td>27468383.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>247394588</td>
<td>10</td>
<td>24739458.8</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Bartlett's test for equal variances: chi2(1) = 1.2287 Prob>chi2 = 0.268

Bartlett test for equality of variances (H_0: \( \sigma_1^2 = \sigma_2^2 \)) p-value = .268

One way analysis of variance test for equality of means (H_0: \( \mu_1 = \mu_2 \)) p-value = .93

Discussion for dose = .01

At this dose, the AUC responses are similarly variable (p=.268) and with non-statistically significantly different means (p=.94)

---

At Dose = .05 mg/ml is there a **COMPOUND effect on AREA UNDER CURVE (AUC)?**

```plaintext
<table>
<thead>
<tr>
<th></th>
<th>Summary of auc</th>
</tr>
</thead>
<tbody>
<tr>
<td>compound</td>
<td>Mean    Std. Dev.</td>
</tr>
<tr>
<td>1</td>
<td>14711.15  4420.1143</td>
</tr>
<tr>
<td>2</td>
<td>18682.85 1358.7584</td>
</tr>
<tr>
<td>Total</td>
<td>16697     3744.5886</td>
</tr>
</tbody>
</table>

Analysis of Variance

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>47323203.4</td>
<td>1</td>
<td>47323203.4</td>
<td>4.43</td>
<td>0.0617</td>
</tr>
<tr>
<td>Within groups</td>
<td>106918174</td>
<td>10</td>
<td>10691817.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>154241378</td>
<td>11</td>
<td>14021943.4</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Bartlett's test for equal variances: chi2(1) = 5.2431 Prob>chi2 = 0.022

Bartlett test for equality of variances (H_0: \( \sigma_1^2 = \sigma_2^2 \)) p-value = .022

One way analysis of variance test for equality of means (H_0: \( \mu_1 = \mu_2 \)) p-value = .06

Discussion for dose = .05

Effects of compound are seen at dose = .05. The variability in AUC responses are statistically significantly greater for compound K1256 (boxes 2 and 5). The differences in means are of marginal significance (p=.06)
At Dose = .25 mg/ml is there a **COMPOUND effect** on AREA UNDER CURVE (AUC)?

<table>
<thead>
<tr>
<th>compound</th>
<th>Summary of auc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>1</td>
<td>21807.65</td>
</tr>
<tr>
<td>2</td>
<td>17024.475</td>
</tr>
<tr>
<td>Total</td>
<td>19416.063</td>
</tr>
</tbody>
</table>

**Analysis of Variance**

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>68636283.6</td>
<td>1</td>
<td>68636283.6</td>
<td>6.17</td>
<td>0.0323</td>
</tr>
<tr>
<td>Within groups</td>
<td>11127124.2</td>
<td>10</td>
<td>11127124.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>179907526</td>
<td>11</td>
<td>16355229.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bartlett's test for equal variances: $\chi^2(1) = 8.5587$  Prob>chi2 = 0.003

Bartlett test for equality of variances ($H_0: \sigma_1^2 = \sigma_2^2$) p-value = .003

One way analysis of variance test for equality of means ($H_0: \mu_1^2 = \mu_2^2$) p-value = .03

**Discussion for dose = .25**

Effects of compound are also seen at dose = .25. The variability in AUC responses are again statistically significantly greater for compound K1256 (boxes 3 and 6). The differences in means are statistically significant (p=.03)

**Remarks**

- **For compound K1256, the differences in the sizes of the boxes (boxes 1, 2, and 3) evidences differences in the variabilities of AUC with dose.**

- **Greater variabilities in AUC are seen for compound K1397 (boxes 4, 5, and 6).**

- **For compound K1256, the shift in the location of the box and whisker plots evidences an upward trend in AUC with increasing dose.**

- **For compound K1397, an upward trend is seen in the comparison of doses .01 and .05; this is followed by a leveling off.**

- **Controlling for dose reveals an effect of compound at dose levels .05 and .25, but not at dose .01.**
5. Univariate Repeated Measures Analysis of Variance

Univariate repeated measures analyses of variance, traditionally, assume the very restrictive variance-covariance structure of compound symmetry.

- If compound symmetry is reasonable, the analysis is straightforward to perform and interpret.

- When compound symmetry is not reasonable, the analysis then requires a multivariate (Section 6) or mixed model approach (Section 7)

This section is an introduction to two univariate repeated measures analyses of variance, both utilizing compound symmetry.

  a. Single Sample Univariate Repeated Measures
  b. K Sample Univariate Repeated Measures.
a. Single Sample Univariate Repeated Measures Analysis of Variance

Example –

(Source: Twisk, 2003, page 26) The following illustrative data set consists of n=6 subjects measured (repeatedly) on T=4 occasions of time.

<table>
<thead>
<tr>
<th>Subject, i</th>
<th>( Y_{i1} )</th>
<th>( Y_{i2} )</th>
<th>( Y_{i3} )</th>
<th>( Y_{i4} )</th>
<th>Mean, ( \bar{Y}_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>29</td>
<td>15</td>
<td>26</td>
<td>25.25</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>28</td>
<td>20</td>
<td>32</td>
<td>26.00</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>20</td>
<td>28</td>
<td>30</td>
<td>23.00</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>34</td>
<td>30</td>
<td>34</td>
<td>34.00</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>29</td>
<td>25</td>
<td>29</td>
<td>27.00</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>28</td>
<td>16</td>
<td>34</td>
<td>27.00</td>
</tr>
<tr>
<td>Mean, ( \bar{Y}_i )</td>
<td>27.00</td>
<td>28.00</td>
<td>22.33</td>
<td>30.83</td>
<td>27.00</td>
</tr>
</tbody>
</table>

Analysis Question –

1. Does response \( Y \) change over time?

Notation –

\( Y_{it} \) = response of subject “i” at time “t”

- **Subjects** are indexed with “i” and range \( i = 1 \ldots N=6 \)
- **Time** will be identified with “t” and range \( t = 1 \ldots T=4 \).
- **Outcome** is represented using \( Y \).

\( Y_{it} \) = response of \( i^{th} \) person on the \( t^{th} \) repeated measure.
Model –
This is actually a mixed model two-way layout, with one factor (TIME) fixed and one factor (SUBJECT) random. Deviation from means parameterization is used.

\[
Y_{it} = \mu + \lambda_t + S_i + (S\lambda)_{it} + e_{it}
\]

- \(\mu\) = overall mean
- \(\lambda_t\) = fixed effect deviation associated with time "t"; \(\sum_{t=1}^{4} \lambda_t = 0\)
- \(S_i\) = random effect that represents the deviation of subject "i"
- \(e_{it}\) = random error
- **Note** -- It is possible for there to exist a (SUBJECT)x(TIME) interaction \(\lambda S\) with variance \(\sigma^2_{\lambda S}\). Here, however, it is NOT estimable because the sample size is only 1 at each combination of (subject) x (time) making it indistinguishable from the random error \(\sigma^2_e\).

It’s helpful to distinguish the systematic versus random terms in the model:

<table>
<thead>
<tr>
<th>Systematic = (\mu_t)</th>
<th>Random = (e_{it})</th>
</tr>
</thead>
<tbody>
<tr>
<td>= (\mu + \lambda_t)</td>
<td>= (S_i + e_{it})</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Overall</th>
<th>Time</th>
<th>Subject</th>
<th>residual error</th>
</tr>
</thead>
</table>
Assumptions –
The following assumptions underly the framework of compound symmetry here.

- $S_i$ is distributed Normal ($0$, $\sigma_S^2$)
- $e_{it}$ is distributed Normal ($0$, $\sigma_e^2$)
- $S_i$ and $e_{it}$ are mutually independent (thus, covariance = 0).
- $S_i$ and $S_{i'}$ are mutually independent for $i \neq i'$ (thus, covariance=0)
- $e_{it}$ and $e_{i't'}$ are mutually independent for $i \neq i'$ (thus, covariance=0)

Mean Structure –
Under deviation from means parameterization we have,

$$E[Y_{it}] = E[\mu] + E[\lambda_t] + E[S_i] + E[e_{it}]$$

$$= \mu + \lambda_t + 0 + 0$$

$$= \mu + \lambda_t$$
**Variance-Covariance Structure –**
Because we are assuming (1) mutual independence of distinct subjects “i” and “i*” and (2) compound symmetry, we need only solve for the variance of \( Y_t \) at each time “t” and the covariance of \( Y_t \) and \( Y_{t'} \) for any pair of time points \( t \neq t' \) within the same individual “i”

\[
\text{Var}[Y_{it}] = \text{Var}[\mu + \lambda_t + S_i + e_{it}]
= \text{Var}[S_i + e_{it}]
= \text{Var}[S_i] + 2\text{Cov}[S_i, e_{it}] + \text{Var}[e_{it}]
= \sigma_S^2 + 0 + \sigma_e^2
= \sigma_S^2 + \sigma_e^2
\]

\[
\text{Cov}[Y_{it}, Y_{it'}] = \text{Cov}[S_i + e_{it}, S_i + e_{it'}]
= \text{Var}[S_i] + \text{Cov}[S_i, e_{it'}] + \text{Cov}[e_{it}, S_i] + \text{Cov}[e_{it}, e_{it'}]
= \sigma_S^2 + 0 + 0 + 0
= \sigma_S^2
\]

*Yielding* \( \text{Var-Cov} [Y_{i1}, Y_{i2}, Y_{i3}, Y_{i4}] = \)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(\sigma_S^2 + \sigma_e^2)</td>
<td>(\sigma_S^2)</td>
<td>(\sigma_S^2)</td>
<td>(\sigma_S^2)</td>
</tr>
<tr>
<td>2</td>
<td>(\sigma_S^2)</td>
<td>(\sigma_S^2 + \sigma_e^2)</td>
<td>(\sigma_S^2)</td>
<td>(\sigma_S^2)</td>
</tr>
<tr>
<td>3</td>
<td>(\sigma_S^2)</td>
<td>(\sigma_S^2)</td>
<td>(\sigma_S^2 + \sigma_e^2)</td>
<td>(\sigma_S^2)</td>
</tr>
<tr>
<td>4</td>
<td>(\sigma_S^2)</td>
<td>(\sigma_S^2)</td>
<td>(\sigma_S^2)</td>
<td>(\sigma_S^2 + \sigma_e^2)</td>
</tr>
</tbody>
</table>
Setting $\sigma^2_s + \sigma^2_e = \sigma^2$ and $\rho = \frac{\sigma^2_s}{\sigma^2_s + \sigma^2_e}$, the structure of compound symmetry here matches that on page 17:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$\sigma^2$</td>
<td>$\rho \sigma^2$</td>
<td>$\rho \sigma^2$</td>
<td>$\rho \sigma^2$</td>
</tr>
<tr>
<td>2</td>
<td>$\rho \sigma^2$</td>
<td>$\sigma^2$</td>
<td>$\rho \sigma^2$</td>
<td>$\rho \sigma^2$</td>
</tr>
<tr>
<td>3</td>
<td>$\rho \sigma^2$</td>
<td>$\rho \sigma^2$</td>
<td>$\sigma^2$</td>
<td>$\rho \sigma^2$</td>
</tr>
<tr>
<td>4</td>
<td>$\rho \sigma^2$</td>
<td>$\rho \sigma^2$</td>
<td>$\rho \sigma^2$</td>
<td>$\sigma^2$</td>
</tr>
</tbody>
</table>

Note - $\rho = \frac{\sigma^2_s}{\sigma^2_s + \sigma^2_e}$ is called the intra-class correlation coefficient.

The Analysis of Variance

Recall the reasoning in the analysis of variance that we learned in Topic 7.

1. The total “corrected” variability is partitioned into its sources.
2. Each “partition” or “source” is an estimate of some variance component (e.g., variance of means of groups defined by group).
3. We obtain the expected values of each variance component. These guide us in the construction of F tests.
4. F tests are performed and are comparisons of variance components. Significant F tests are evidence of significant differences in the means of interest.

The partitioning of the variance is a little more involved when the analysis of variance design includes a repeated measures factor. This is because the sources of variation are no longer independent.

1. Start with the partition corresponding to a simple one way anova (that is, pretend that you have independent groups defined by TIME).

$$SSQ_{\text{Total,corrected}} = SSQ_{\text{TIME}} + SSQ_{\text{ERROR}}$$

$$\sum_{i=1}^{N} \sum_{t=1}^{T} (Y_{it} - \bar{Y}_t)^2 = \sum_{i=1}^{N} \sum_{t=1}^{T} (\bar{Y}_i - \bar{Y})^2 + \sum_{i=1}^{N} \sum_{t=1}^{T} (Y_{it} - \bar{Y}_t)^2$$

$$\text{Df} = NT - 1 \quad \text{Df} = (T-1) \quad \text{Df} = T(N-1)$$
(2) Set aside the SSQ\textsubscript{TIME}. It represents the source of variability attributable to the variability among the means at each time, the "main effects of TIME".

(3) Consider the SSQ\textsubscript{ERROR}. Partition this. Specifically, from this error, we remove the source of variability attributable to the variability among the means of each subject (the "main effects of SUBJECT").

\[
\text{SSQ}_{\text{ERROR}} = \text{SSQ}_{\text{SUBJECT}} + \text{SSQ}_{\text{ERROR WITHIN TIME}}
\]

\[
\sum_{i=1}^{N} \sum_{t=1}^{T} (Y_{it} - \overline{Y}_{i.})^2 = \sum_{i=1}^{N} \sum_{t=1}^{T} (\overline{Y}_{.t} - \overline{Y}.)^2 + \sum_{i=1}^{N} \sum_{t=1}^{T} (Y_{it} - \overline{Y}_{.t} - \overline{Y}_{i.} + \overline{Y}.)^2
\]

\[
\text{Df} = T(N-1) \quad \text{Df} = (N-1) \quad \text{Df} = (T-1)(N-1)
\]

Thus, you have the following schematic for the partitioning of the total variance

\[
\text{SSQ}_{\text{Total,corrected}} = \text{SSQ}_{\text{TIME}} + \text{SSQ}_{\text{ERROR}}
\]

\[
\text{SSQ}_{\text{SUBJECT}} + \text{SSQ}_{\text{ERROR WITHIN TIME}}
\]

Here is the partitioning of the Total Sum of Squares, corrected (SSQ\textsubscript{Total,corrected})

\[
\sum_{i=1}^{N} \sum_{t=1}^{T} (Y_{it} - \overline{Y}.)^2 = \sum_{i=1}^{N} \sum_{t=1}^{T} (\overline{Y}_{.t} - \overline{Y}.)^2 + \sum_{i=1}^{N} \sum_{t=1}^{T} (Y_{it} - \overline{Y}_{.t} - \overline{Y}_{i.} + \overline{Y}.)^2
\]

And here is the partitioning of the total Degrees of Freedom, corrected:

\[
NT-1 = (T-1) + T(N-1)
\]

\[
(N - 1) + (T-1)(N-1)
\]
Mean Squares/Variance Components and their Expected Values

We’re not quite ready to fill in an analysis of variance table. We need to see the expected values of the variance components that we’ve just extracted.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SSQ</th>
<th>E[Mean Square]</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIME (T-1)</td>
<td>T</td>
<td>[\sum_{i=1}^{N} \sum_{t=1}^{T} (\bar{Y}<em>{it} - \bar{Y}</em>{..})^{2}]</td>
<td>(\sigma_{e}^{2} + \frac{N \sum_{i=1}^{T} \lambda_{i}^{2}}{(T-1)})</td>
</tr>
<tr>
<td>SUBJECTS (N-1)</td>
<td>N</td>
<td>[\sum_{i=1}^{N} \sum_{t=1}^{T} (\bar{Y}<em>{i..} - \bar{Y}</em>{..})^{2}]</td>
<td>(\sigma_{e}^{2} + T \sigma_{s}^{2})</td>
</tr>
<tr>
<td>ERROR WITHIN TIME (T-1)(N-1)</td>
<td>T</td>
<td>[\sum_{i=1}^{N} \sum_{t=1}^{T} (Y_{it} - \bar{Y}<em>{i..} - \bar{Y}</em>{..} + \bar{Y}_{..})^{2}]</td>
<td>(\sigma_{e}^{2})</td>
</tr>
</tbody>
</table>

Reminder – The variance due to subject x time interaction cannot be estimated when the number of observations at each combination of subject x time =1 and so is absorbed in the error within time variance.

Analysis of Variance Table

The expected mean squares above suggests a slight re-organization of the analysis of variance table when the design includes a repeated measures factor (here, TIME).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SSQ</th>
<th>E[Mean Square]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between SUBJECTS</td>
<td>N</td>
<td>[\sum_{i=1}^{N} \sum_{t=1}^{T} (\bar{Y}<em>{i..} - \bar{Y}</em>{..})^{2}]</td>
<td>(\sigma_{e}^{2} + T \sigma_{s}^{2})</td>
</tr>
<tr>
<td>SUBJECTS (N-1)</td>
<td>T</td>
<td>[\sum_{i=1}^{N} \sum_{t=1}^{T} (\bar{Y}<em>{i..} - \bar{Y}</em>{..})^{2}]</td>
<td>(\sigma_{e}^{2} + \frac{N \sum_{i=1}^{T} \lambda_{i}^{2}}{(T-1)})</td>
</tr>
<tr>
<td>Within SUBJECTS</td>
<td>T</td>
<td>[\sum_{i=1}^{N} \sum_{t=1}^{T} (\bar{Y}<em>{i..} - \bar{Y}</em>{..})^{2}]</td>
<td>(\sigma_{e}^{2} + \frac{N \sum_{i=1}^{T} \lambda_{i}^{2}}{(T-1)})</td>
</tr>
<tr>
<td>ERROR (TIME) (T-1)(N-1)</td>
<td>T</td>
<td>[\sum_{i=1}^{N} \sum_{t=1}^{T} (Y_{it} - \bar{Y}<em>{i..} - \bar{Y}</em>{..} + \bar{Y}_{..})^{2}]</td>
<td>(\sigma_{e}^{2})</td>
</tr>
<tr>
<td>TOTAL, corrected NT-1</td>
<td>N</td>
<td>[\sum_{i=1}^{N} \sum_{t=1}^{T} (Y_{it} - \bar{Y}_{..})^{2}]</td>
<td></td>
</tr>
</tbody>
</table>

Hypothesis tests
Recall that the analysis question was “Does response Y change over time?”

From the partition and solution for expected mean squares, this can now be extracted as

\[
F = \frac{\text{mean square [TIME]}}{\text{mean square [ERROR(TIME)]}}
\]

\[
\text{Df} = (T-1), (T-1)(N-1)
\]

Rejection of the null hypothesis of “no effect of TIME” is suggested by large values of this F statistic.

**Assessment of the Assumption of Compound Symmetry**

Violation of compound symmetry can be a problem.

Violation of the assumption of compound symmetry affects the validity of the F test for assessing the effect of repeated measures occasion (in this example, “time”):

- The F test no longer has df = (T-1), (T-1)(N-1). The correct df are smaller.
- When compound symmetry is violated and the F test is used anyway, the actual type I error is larger than desired (read: the F test used will reject the null hypothesis too often when compound symmetry is violated).

It is possible to test the reasonableness of compound symmetry but, actually, the hypothesis test is of sphericity.

- **Compound symmetry** and **sphericity** are similar but not the same. Sphericity is more general.
  - Whereas in **compound symmetry**, the variance of outcome at each occasion is constant (and equal to \( \sigma_s^2 + \sigma_e^2 = \sigma^2 \)) and the covariance of any pair of repeated measures outcome is also constant (and equal to \( \rho = \sigma_s^2/(\sigma_s^2 + \sigma_e^2) \)),
  - **sphericity** means that the variance of the difference between any pair of repeated measures outcomes is a constant, that is \( \text{Var} [Y_t - Y_{t'}] = \text{constant for all } t \neq t' \)
- The univariate repeated measures F tests are valid under the more general assumption of sphericity.

Mauchley’s test of sphericity is a chi square test.
- Look for non-significant (small) values of Mauchley’s test as support of the assumption of sphericity.

- Large Mauchley test statistic values suggest violation of sphericity.

- **Beware:** For small sample sizes, Mauchley’s test might fail to reject sphericity when it should (type II error).

**Adjustment of the F test for Departure from Compound Symmetry**

For modest violations of sphericity, the univariate repeated measures F tests can still be used but should now incorporate adjustment to the degrees of freedom.

The adjusted degrees of freedom is downward.

Under non-sphericity, the F test rejects too many true null hypotheses. Adjusting the degrees of freedom downward makes it more difficult to reject a true null hypothesis; thus, type I error is reduced.

The extent of adjustment is based on a parameter called epsilon $\varepsilon$.

Epsilon $\varepsilon$ is a measure of the extent of departure from sphericity.

When sphericity holds, $\varepsilon = 1$

DF for F test = $(T-1), (T-1)(N-1)$

When sphericity does NOT hold, $\varepsilon < 1$

A better DF for F test = $\varepsilon(T-1), \varepsilon(T-1)(N-1)$

Among the available adjustments of degrees of freedom are the following three.

1. **LOWER BOUND** is MOST CONSERVATIVE.
   - Uses $DF = 1, (T-1)$
   - This is sometimes “too conservative”; it sometimes fails to reject the null when it should. This is a type II error and corresponds to an analysis with too little power.

2. **GREENHOUSE -GEISSER** is intermediate
   - Uses $DF = \varepsilon(T-1), \varepsilon(T-1)(N-1)$ where $\varepsilon$ has been estimated from the variance-covariance of the data. This can also be “too conservative” sometimes. If so, the Hunyh-Feldt adjustment might be more appropriate

3. **HUNYH-FELDT** is LEAST CONSERVATIVE
   - Uses $DF = \varepsilon(T-1) \varepsilon(T-1)(N-1)$
   - When $\varepsilon > .75$ and $N < 2T$, consider the Hunyh-Feldt correction.

**Tip** –
Since Mauchley’s test may not detect small departures from sphericity, and since these can be a problem, it is recommended that F tests in univariate repeated measures designs incorporate adjustments to degrees of freedom.

Illustration - continued

The following illustrates the analysis in SAS

Read in the data into both WIDE and LONG format

```sas
data wide;
  input studyid y1 y2 y3 y4;
cards;
  1 31 29 15 26
  2 24 28 20 32
  3 14 20 28 30
  4 38 34 30 34
  5 25 29 25 29
  6 30 28 16 34
; run;
quit;
data long;
set wide;
  y=y1; day=1; output;
  y=y2; day=2; output;
  y=y3; day=3; output;
  y=y4; day=4; output;
  drop y1 y2 y3 y4;
run;
quit;
proc print data=wide;
title "Data in WIDE format";
run;
quit;
proc print data=long;
title "Data on LONG format";
run;
quit;
```

Plot individual profiles. Save as “jpeg” image
proc gplot data=long;
  plot y*day=studyid/nolegend skipmiss; /* notice use of id */
  title "Single Sample Repeated Measures";
  title2 "Individual Profiles";
run;
quit;

Plot mean profile over time.
goptions reset=symbol;

2010
symbol i=stdlj l=1; /* produce SD bars and join */
proc gplot data=long;
    plot y*day;
    title "Single Sample Repeated Measures";
    title "Mean Profile +/- 1 Standard Deviation";
run;
quit;

Mean Profile +/- 1 Standard Deviation

Descriptives on means and variances and variance-covariance matrix
Note – these do appear in the output for repeated measures anova later, too
### Single Sample Repeated Measures

#### Means and Variances Over Time

The MEANS Procedure

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>y1</td>
<td>6</td>
<td>27.0000000</td>
<td>65.6000000</td>
</tr>
<tr>
<td>y2</td>
<td>6</td>
<td>28.0000000</td>
<td>20.4000000</td>
</tr>
<tr>
<td>y3</td>
<td>6</td>
<td>22.3333333</td>
<td>39.4666667</td>
</tr>
<tr>
<td>y4</td>
<td>6</td>
<td>30.8333333</td>
<td>9.7666667</td>
</tr>
</tbody>
</table>

*These variances are quite Unequal!!*

#### Covariance Matrix, DF = 5

<table>
<thead>
<tr>
<th></th>
<th>y1</th>
<th>y2</th>
<th>y3</th>
<th>y4</th>
</tr>
</thead>
<tbody>
<tr>
<td>y1</td>
<td>65.600000000</td>
<td>34.400000000</td>
<td>-7.200000000</td>
<td>7.200000000</td>
</tr>
<tr>
<td>y2</td>
<td>34.400000000</td>
<td>20.400000000</td>
<td>-0.800000000</td>
<td>3.800000000</td>
</tr>
<tr>
<td>y3</td>
<td>-7.200000000</td>
<td>-0.800000000</td>
<td>39.46666667</td>
<td>5.46666667</td>
</tr>
<tr>
<td>y4</td>
<td>7.200000000</td>
<td>3.800000000</td>
<td>5.46666667</td>
<td>9.76666667</td>
</tr>
</tbody>
</table>

*Note:*
1. Along the diagonal, you can see the variances that were obtained previously.
2. Elsewhere, off-diagonal, you can see that the covariances are quite unequal too!

PROC GLM can be used to get a Levene test of homogeneity of variance.

```
proc glm data=long;
    class day;
```
model y=day;
means day/hovtest; /* option hov is test of homogeneity of var */
run;
quit;

The GLM Procedure

Levene's Test for Homogeneity of y Variance
ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Squares</th>
<th>Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>day</td>
<td>3</td>
<td>7502.1</td>
<td>2500.7</td>
<td>1.55</td>
<td>0.2331</td>
</tr>
<tr>
<td>Error</td>
<td>20</td>
<td>32305.4</td>
<td>1615.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

We see that Levene’s test of homogeneity of variance (a one way anova of a dispersion random variable) is not performing well in detecting the heterogeneity of variance.

PROC GLM can be used to get a repeated measures anova under compound symmetry and with test of sphericity.

Note – The output will include results of both the univariate repeated measures anova plus a multivariate repeated measures (discussed in the next section). Below is the portion for the univariate repeated measures anova only

proc glm data=wide;
  class studyid;
  model y1 y2 y3 y4= /nouni;            "nouni” suppresses some descriptives
  repeated time 4/printe;               "printe” produces test of sphericity"
run;
quit;

The GLM Procedure
Repeated Measures Analysis of Variance

Sphericity Tests

<table>
<thead>
<tr>
<th>Variables</th>
<th>DF</th>
<th>Mauchly’s Criterion</th>
<th>Chi-Square</th>
<th>Pr &gt; ChiSq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transformed Variates</td>
<td>5</td>
<td>0.1262513</td>
<td>7.703068</td>
<td>0.1734</td>
</tr>
<tr>
<td>Orthogonal Components</td>
<td>5</td>
<td>0.1115167</td>
<td>8.164995</td>
<td>0.1474</td>
</tr>
</tbody>
</table>

With such a small N (=6), Mauchley’s test, not surprisingly, failed to reject. Thus, it was not very useful.
Repeated Measures Analysis of Variance
Univariate Tests of Hypotheses for Within Subject Effects

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
<th>Adj Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>3</td>
<td>224.7916667</td>
<td>74.9305556</td>
<td>2.81</td>
<td>0.0752</td>
<td>0.1311</td>
</tr>
<tr>
<td>Error(time)</td>
<td>15</td>
<td>399.9583333</td>
<td>26.6638889</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Greenhouse-Geisser Epsilon    0.4857
Huynh-Feldt Epsilon           0.6343

Notes - (1) The greenhouse-geisser and Hunyh-Feldt epsilons (0.4857 and 0.6343) are quite a bit less than ONE, consistent with the lack of sphericity we saw earlier
(2) The p-value .0752 for the Unadjusted F value is, therefore, not appropriate.
(3) The Greenhouse-Geisser and Hunyh-Feldt p-values of .1311 and .1114 being less significant correspond to use of downward adjusted degrees of freedom values.

PROC MIXED can also be used to obtain a univariate repeated measurements analysis of variance. It uses data in LONG format. More on PROC MIXED later...

```
proc mixed data=long;
  class studyid day;
  model y=day;
  repeated day/subject=studyid type=cs;  /* cs - "compound symmetric"
run;
quit;
```

The Mixed Procedure
Type 3 Tests of Fixed Effects

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>day</td>
<td>3</td>
<td>15</td>
<td>2.81</td>
<td>0.0752</td>
</tr>
</tbody>
</table>

Notes - (1) The unadjusted F=2.81 and p-value of 0.0752 match PROC GLM overall F and p-value
(2) PROC MIXED does not provide the greenhouse-geisser nor hunyh-feldt estimates of epsilon nor downward adjusted p-values.
(3) The advantage of PROC MIXED is that it allows you to explore and assess the goodness of fit of a variety of variance-covariance structures. This is not possible in PROC GLM.

Stata v 10.1 Illustration
The following will also work in Stata v 11
. *---------- data are in long format ------*
. use "/Users/carolbigelow/Desktop/unit8page39_long.dta"

. *---------- descriptives on means and variances at each time point t ---*
. table time, contents(mean yscore sd yscore)

<table>
<thead>
<tr>
<th>time</th>
<th>mean(yscore)</th>
<th>sd(yscore)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>8.099382</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>4.516636</td>
</tr>
<tr>
<td>3</td>
<td>22.3333</td>
<td>6.28225</td>
</tr>
<tr>
<td>4</td>
<td>30.8333</td>
<td>3.125167</td>
</tr>
</tbody>
</table>

. *------- variance-covariance matrix ------*
. * data must be in wide format *
. preserve
. reshape wide yscore, i(subject) j(time)

(note: j = 1 2 3 4)

Data                               long   ->   wide
-------------------------------------------------------------------------------
Number of obs.                       24   ->       6
Number of variables                   3   ->       5
j variable (4 values)              time   ->   (dropped)
xij variables:                        yscore -> yscore1 yscore2 ... yscore4
-------------------------------------------------------------------------------

. *----- variance-covariance matrix ---*
. correlate yscore1 yscore2 yscore3 yscore4, cov
. *--------  test of equality of variances ----*
. *---- data in long format ---*
. restore
. sort time
. robvar yscore, by (time)

<table>
<thead>
<tr>
<th>Summary of yscore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean  Std. Dev.  Freq.</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

W0  =  1.8617304   df(3, 20)     Pr > F = 0.16862176
W50 =  1.8325496   df(3, 20)     Pr > F = 0.17374147
W10 =  1.8617304   df(3, 20)     Pr > F = 0.16862176

Note – W0, W50 and W10 are variations on Levene’s test for homogeneity of variance. As in SAS, they all fail to reject Ho.
. anova yscore subject time, repeated(time)

    Number of obs =  24     R-squared =  0.5561
    Root MSE = 5.16371     Adj R-squared =  0.3193

Source | Partial SS    df       MS           F     Prob > F
---------+----------------------------------------------------
Model |         501     8      62.625       2.35     0.0733
|                     
subject |  276.208333     5  55.2416667       2.07     0.1260
time |  224.791667     3  74.9305556       2.81     0.0752
|                     
Residual |  399.958333    15  26.6638889
---------+----------------------------------------------------
Total |  900.958333    23  39.1721014

Between-subjects error term:  subject
Levels:  6         (5 df)
Lowest b.s.e. variable:  subject

Repeated variable:  time

          Huynh-Feldt epsilon        =  0.6343
          Greenhouse-Geisser epsilon =  0.4857
          Box’s conservative epsilon =  0.3333

Source |     df      F    Regular    H-F      G-G      Box
---------+----------------------------------------------------
time |      3     2.81   0.0752   0.1114   0.1311   0.1545
Residual |     15
---------+----------------------------------------------------

Note – We get the same values as SAS for the Huynh-Feldt and Greenhouse-Geisser epsilon. Thus, the p-value for the unadjusted F (.0752) for the effect of time is thus inappropriate.
b. K Sample Univariate Repeated Measures Analysis of Variance

Example –

(source: Poththoff and Roy) Recall the two group growth curve data for boys and girls introduced on page 6. Recall that this was a study of dental growth in two groups of children, 11 girls and 16 boys and that for each child there are four repeated measurements, equally spaced.

<table>
<thead>
<tr>
<th>Subject, i</th>
<th>Age in years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Girls</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Boys</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>27</td>
</tr>
</tbody>
</table>

There are two analysis questions

2. Is there a difference between girls and boys?

3. Is there a difference between girls and boys with respect to change?

This is a mixed model three-way layout, with two factors fixed and one factor random. Note - In section 6 (Introduction to Linear Mixed Models), we’ll develop a framework for thinking about the mixed model). Here, focus is on analysis of variance framework and the ideas of compound symmetry.

- Gender is a fixed factor at 2 levels: girl and boy.
- Within each gender, subjects are a random sample of 11 girls from a population of girls and a random sample of 16 boys from a population of boys.
- Time is a fixed repeated measures factor, at 4 levels: 8, 10, 12, and 14, equally spaced.
- There is one measurement of dental growth per child at each of the four occasions of time.
Notation

\[ Y_{hit} = \text{dental growth at time “t” on child “i” in group “h”} \]

- **Subjects** are indexed with “i” and will range \( i = 1 \ldots n_j \)
- **Group (boys v girls)** are indexed with “h” and will range \( h = 1, 2 \)
- **Time** will be identified with “t” and will range \( t = 1 \ldots 4 \).
- **Outcome = dental growth** will be represented using \( Y \).

Model Definition

\[ Y_{hit} = \mu + \tau_h + \lambda_t + (\tau\lambda)_{ht} + S_{hi} + e_{hit} \]

- \( \mu \) = overall mean
- \( \tau_h \) = deviation from overall mean associated with being in group "h"
- \( \lambda_t \) = deviation associated with time "t"
- \( (\tau\lambda)_{ht} \) = additional deviation associated with group "h" and time "t" in combination (interaction)
- \( S_{hi} \) = random effect that represents the deviation of subject "i" in group "h"
- \( e_{hit} \) = random deviation (possibly due to more than measurement error)
Here are the **systematic** versus **random** terms in this two group model:

\[
Y_{hit} = \mu + \tau_t + \lambda_i + (\tau\lambda)_{hit} + S_{hi} + e_{hit}
\]

<table>
<thead>
<tr>
<th>Gender</th>
<th>Time</th>
<th>Subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systematic = (\mu_{hit}) = (\mu + \tau_t + \lambda_i + (\tau\lambda)_{hit})</td>
<td>Random = (e_{hit}) = (S_{hi} + e_{hit})</td>
<td></td>
</tr>
</tbody>
</table>

The **Univariate Repeated Measures Analysis of Variance Normal Distribution Theory** Model and Assumptions are now the following.

- \(E[ Y_{hit} ] = \mu_{hit} = \mu + \tau_t + \lambda_i + (\tau\lambda)_{hit}\)
- \(S_{hi}\) is distributed Normal \((0, \sigma^2_s)\)
- \(e_{hit}\) is distributed Normal \((0, \sigma^2_e)\)
- \(S_{hi}\) and \(e_{hit}\) are mutually independent.
- \(S_{hi}\) and \(S_{hit}\) are mutually independent
- \(e_{hit}\) and \(e_{hat}\) are mutually independent
6. **Introduction to Linear Mixed Model Methods**