

BIOS 6612

Lecture 14

Repeated Measures ANOVA

Additional Reading:
KKMN Section 26.4.5

Review (Lecture 13)/ Current (Lecture 14)/ Preview (Lecture 15)

- Lecture 13: Linear Mixed Models
 - 2 time points per persons
 - Paired t-test
 - Linear regression
 - Random intercept model
 - Notation
 - Linear mixed models with a random intercept
 - Linear mixed models specifying the covariance structure
- Lecture 14: Repeated Measures ANOVA
 - ANOVA for repeated measurements
- Lecture 15: Modelling the Random Effects

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{b} + \boldsymbol{\varepsilon} \text{ and } \mathbf{V} = \text{Var}(\mathbf{Y}) = \mathbf{Z}\mathbf{G}\mathbf{Z}' + \mathbf{R}$$

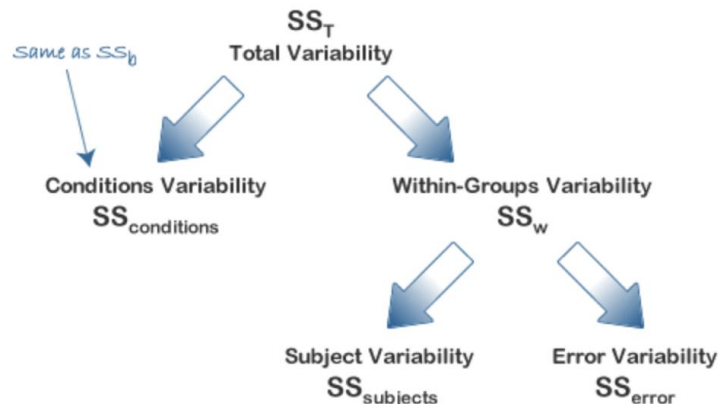
- Specify the random effect \mathbf{b}
- Random intercept, random slope

RMANOVA

- Repeated measures ANOVA is the equivalent of the one-way ANOVA, but for **related, not independent groups**
 - Test to detect any overall differences between related means
- ANOVA partitions total variability (SS_T) into between-groups variability (SS_b) and within-groups variability (SS_w)



- In this design, **within-group variability (SS_w) is defined as the error variability (SS_{error})**
 - $F \text{ statistic} = MS_{\text{model}} / MS_{\text{error}} = MS_b / MS_w$
- RMANOVA can **further partition this error term**, reducing its size



REVIEW: One-Way ANOVA

Respiratory Quotient (RQ), which measures the ratio between O₂ consumed and CO₂ produced, was measured for 15 participants after a week of consuming one of three diets of varying diet compositions.

	<i>Standard Diet</i> (<i>n</i> = 5)	<i>High Fat Diet</i> (<i>n</i> = 5)	Low Fat Diet (n = 5)
	0.80	0.77	0.83
	0.78	0.77	0.82
	0.82	0.80	0.87
	0.76	0.73	0.79
	0.83	0.83	0.86
Mean	0.798	0.780	0.834
Variance	0.00082	0.0014	0.00103

$\bar{Y} = 0.804$
 $SD(Y) = 0.03832$

$$SS_{\text{Total}} = 14 \times (0.03832)^2 = 0.02056$$

$$SS_{\text{Diet}} = 5 \times [(0.798 - 0.804)^2 + (0.780 - 0.804)^2 + (0.834 - 0.804)^2] = 0.00756$$

$$SS_{\text{Error}} = SS_{\text{Total}} - SS_{\text{Diet}} = 0.02056 - 0.00756 = 0.013$$

$$MS_{\text{Error}} = [(0.00082) + (0.0014) + (0.00103)] / 3 = 0.00108333 = s_p^2$$

# diets-1=3-1=2	$df_{\text{error}}=df_{\text{total}}-df_{\text{model}}=14-2=12$	# subjects-1=15-1=14	$F=MS_{\text{model}}/MS_{\text{Error}}$
-----------------	---	----------------------	---

Source	Sum of Squares	d.f.	Mean Square	F	p-value
Model	0.00756	2	0.00378	3.489	0.0639
Error	0.013	12	0.00108333		
Total	0.02056	14			



When n are equal:

Estimate of $MS_{\text{Error}} = \overline{\text{VAR}}$
 $= (0.001030 + 0.00082 + 0.0014) / 3 = 0.00108333$

For unequal n, MS_{Error} is a weighted average of the variances, with weights $(n_k - 1)$.

$H_0: \mu_{\text{standard}} = \mu_{\text{high}} = \mu_{\text{low}}$
 RQ does not differ significantly across the three diets.

$$MS_{\text{Error}} = SS_{\text{Error}} / df_{\text{Error}}$$

	<i>Low Fat</i> (n = 5)	<i>Standard Fat</i> (n = 5)	High Fat (n = 5)
Mean	0.834	0.798	0.780
Variance	0.00103	0.00082	0.0014



One-Way ANOVA Results

The GLM Procedure

Class Level Information

Class	Levels	Values
diet	3	1 2 3

Number of Observations Read	15
Number of Observations Used	15

The GLM Procedure

Dependent Variable: rq

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00756000	0.00378000	3.49	0.0639
Error	12	0.01300000	0.00108333		
Corrected Total	14	0.02056000			

R-Square	Coeff Var	Root MSE	rq Mean
0.367704	4.093785	0.032914	0.804000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
diet	2	0.00756000	0.00378000	3.49	0.0639

Source	DF	Type III SS	Mean Square	F Value	Pr > F
diet	2	0.00756000	0.00378000	3.49	0.0639

```
PROC GLM DATA=rq2;  
  CLASS diet;  
  MODEL rq = diet;  
RUN;
```

← $H_0: \mu_{\text{standard}} = \mu_{\text{high}} = \mu_{\text{low}}$
RQ does not differ significantly
between the three diets.

One-way ANOVA: Results from R

```

respquot <- data.frame(subject=1:5,
                        standard=c(.8,.78,.82,.76,.83),
                        high.fat=c(.77,.77,.80,.73,.83),
                        low.fat=c(.83,.82,.87,.79,.86))
# put the data in "long" format
respquot.long <- reshape(respquot,direction='long',
                          varying=2:4,
                          v.names='rq',
                          idvar='subject',
                          times=colnames(respquot)[-1],
                          timevar='diet')
# simple one-way ANOVA, ignoring repeated measures
mod1 <- lm(rq ~ diet,data=respquot.long)
anova(mod1)

## Analysis of Variance Table
##
## Response: rq
##          Df Sum Sq  Mean Sq F value Pr(>F)
## diet      2 0.00756  0.0037800   3.4892 0.0639 .
## Residuals 12 0.01300  0.0010833
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

Repeated Measures Data

Repeated Measures Data: The same outcome/response is measured on the same individual under multiple conditions (or at multiple times) or the same outcome/response is measured for clusters or groups of similar individuals.

Example: Respiratory Quotient (RQ), which measures the ratio between O₂ consumed and CO₂ produced, was measured for 5 subjects after a week of consuming one of three diets of varying diet compositions (with each diet separated by a week “washout” period).

<i>Subject</i>	<i>Standard Diet</i>	<i>High Fat Diet</i>	<i>Low Fat Diet</i>	<i>Subject Mean</i>
1	0.80	0.77	0.83	0.80
2	0.78	0.77	0.82	0.79
3	0.82	0.80	0.87	0.83
4	0.76	0.73	0.79	0.76
5	0.83	0.83	0.86	0.84
Diet Mean	0.798	0.780	0.834	0.804
Variance	0.00082	0.0014	0.00103	

Design: Rather than recruit 15 individuals (5 subjects for each diet), we could recruit 5 individuals and have each individual receive each of the three diets.

Benefits:

- Fewer participants needed
- Between-Subject variability is removed from error
 - More powerful

Drawbacks:

- Participants may be more likely to drop from the study
 - Missing values result in deletion of the subject when using traditional RM ANOVA approaches.
- Carry-over effects or order-effects possible.

Assumptions for Repeated Measures ANOVA

- **Normality:** Each population is normally distributed, and thus the errors follow a normal distribution.
- **Homogeneity of variances:** The variances of the k populations/treatments are the same.
- **Independence of experimental units:** Experimental units (e.g., individuals or clusters) are independent
 - Account for the correlation between measurements within individuals or clusters
- **Homogeneity of covariances:** Equal correlation between conditions


The join assumption of homogeneity of variances and of covariances is known as *compound symmetry* or *sphericity*.

- **Mauchly's test** is one method for testing the assumption of sphericity.
- There are two common measures of sphericity:
 - Greenhouse-Geisser (conservative)
 - Huynh-Feldt (liberal)

Remedy: If the assumption of sphericity is violated:

- 1) The F statistic df can be corrected by multiplying by one of the sphericity estimates
- 2) Multivariate approach can be used which does not require this assumption

One-Way Repeated Measures ANOVA in Matrix Form

For $i=1,\dots,5$, $E[Y_i] = \beta_s \text{standard}_i + \beta_{HF} \text{highfat}_i + \beta_{LF} \text{lowfat}_i$ 

And $E[\mathbf{Y}] = \mathbf{X}\boldsymbol{\beta}$ where

$$\mathbf{Y} = \begin{bmatrix} Y_{11} \\ Y_{12} \\ Y_{13} \\ Y_{21} \\ Y_{22} \\ Y_{23} \\ Y_{31} \\ Y_{32} \\ Y_{33} \\ Y_{41} \\ Y_{42} \\ Y_{43} \\ Y_{51} \\ Y_{52} \\ Y_{53} \end{bmatrix} = \begin{bmatrix} 0.80 \\ 0.77 \\ 0.83 \\ 0.78 \\ 0.77 \\ 0.82 \\ 0.82 \\ 0.80 \\ 0.87 \\ 0.76 \\ 0.73 \\ 0.79 \\ 0.83 \\ 0.83 \\ 0.86 \end{bmatrix} \text{ and } \mathbf{X}\boldsymbol{\beta} = \begin{bmatrix} 100 \\ 010 \\ 001 \\ 100 \\ 010 \\ 001 \\ 100 \\ 010 \\ 001 \\ 100 \\ 010 \\ 001 \\ 100 \\ 010 \\ 001 \end{bmatrix} \begin{bmatrix} \beta_s \\ \beta_{HF} \\ \beta_{LF} \end{bmatrix} \text{ OR } = \begin{bmatrix} Y_{11} \\ Y_{12} \\ Y_{13} \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ Y_{51} \\ Y_{52} \\ Y_{53} \end{bmatrix} = \begin{bmatrix} 0.80 \\ 0.77 \\ 0.83 \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ 0.83 \\ 0.83 \\ 0.86 \end{bmatrix} \text{ and } \mathbf{X}\boldsymbol{\beta} = \begin{bmatrix} 100 \\ 010 \\ 001 \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ 100 \\ 010 \\ 001 \end{bmatrix} \begin{bmatrix} \beta_s \\ \beta_{HF} \\ \beta_{LF} \end{bmatrix}$$

```
DATA RQ2;
INPUT id diet rq;
DATALINES;
1 1 .80
1 2 .77
1 3 .83
2 1 .78
2 2 .77
2 3 .82
3 1 .82
3 2 .80
3 3 .87
4 1 .76
4 2 .73
4 3 .79
5 1 .83
5 2 .83
5 3 .86
;
```

\mathbf{X} is 15x3 matrix of full rank

Sphericity Assumption Implies: $\text{Var}(\mathbf{Y})$ is a 15x15 matrix such that

[illegible]

$$Var(Y) = \begin{pmatrix} \sigma^2 + \sigma_\varepsilon^2 & \sigma^2 & \sigma^2 & & & & 0 \\ \sigma^2 & \sigma^2 + \sigma_\varepsilon^2 & \sigma^2 & & & & \\ \sigma^2 & \sigma^2 & \sigma^2 + \sigma_\varepsilon^2 & & & & \\ & & & \ddots & & & \\ & & & & \sigma^2 + \sigma_\varepsilon^2 & \sigma^2 & \sigma^2 \\ & & & & \sigma^2 & \sigma^2 + \sigma_\varepsilon^2 & \sigma^2 \\ 0 & & & & \sigma^2 & \sigma^2 & \sigma^2 + \sigma_\varepsilon^2 \end{pmatrix}$$

One-Way Repeated Measures ANOVA

	<i>Standard Diet</i>	<i>High Fat Diet</i>	<i>Low Fat Diet</i>	Mean
<i>Subject</i>				
1	0.80	0.77	0.83	0.80
2	0.78	0.77	0.82	0.79
3	0.82	0.80	0.87	0.83
4	0.76	0.73	0.79	0.76
5	0.83	0.83	0.86	0.84
Mean	0.798	0.780	0.834	0.804
Variance	0.00082	0.0014	0.00103	

Treat subject as a random factor.
We now have a two-way ANOVA
(with factors subject and diet).

$$SD(Y) = 0.03832$$

$$SS_{\text{Total}} = 14 \times (0.03832)^2 = 0.02056$$

$$SS_{\text{Diet}} = 5 \times [(0.798 - 0.804)^2 + (0.780 - 0.804)^2 + (0.834 - 0.804)^2] = 0.00756$$

$$SS_{\text{Subject}} = 3 \times [(0.80 - 0.804)^2 + (0.79 - 0.804)^2 + (0.83 - 0.804)^2 + (0.76 - 0.804)^2 + (0.84 - 0.804)^2] = 0.01236$$

$$SS_{\text{Error}} = (.80 - .80 - .798 + .804)^2 + (.77 - .80 - .780 + .804)^2 + (.83 - .80 - .834 + .804)^2 + \dots + (.86 - .84 - .834 + .804)^2 = 0.00064$$

$$SS_{\text{Error}} = SS_{\text{Total}} - SS_{\text{Diet}} - SS_{\text{Subject}} = 0.02056 - 0.00756 - 0.01236 = 0.00064$$

Variability between subjects is removed from the Error!

SS_{Error} is also the Subject \times Diet interaction.

We must assume there is no interaction and this quantity is all error.

$$SS_{\text{Error}} = SS_{\text{Subject} \times \text{Diet}}$$

Repeated Measures ANOVA

Source	Sum of Squares	d.f.	Mean Square	F	p-value
Between Subjects	0.01236	4	0.00309	38.63	<.0001
Within Subjects	[0.0082]	[10]			
Diet	0.00756	2	0.00378	47.25	<.0001
Error	0.00064	8	0.00008		
Total	0.02056	14			

RQ differs significantly across the three diets.

$$MS_{\text{Error}} = \overline{\text{VAR}} - \overline{\text{COV}}$$

MSE has fewer d.f. for repeated measures design (8) compared to independent subjects design (12)

SS(Error) for independent subjects design is equal to SS(Between) + SS(Error) in RM design ($0.013 = 0.01236 + 0.00064$)

Repeated Measures ANOVA with R: Two-factor ANOVA

- Can treat subject as the second factor
- Two-way ANOVA
 - Factor subject varies between subjects
 - Factor diet varies within subjects (comparisons are made within independent units)
 - No interaction term included

```
mod2 <- lm(rq ~ as.factor(subject) + # between subjects
           diet, # within subjects
           data=respquot.long)
anova(mod2)
```

```
## Analysis of Variance Table
##
## Response: rq
##
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
as.factor(subject)	4	0.01236	0.00309	38.625	2.821e-05 ***
diet	2	0.00756	0.00378	47.250	3.711e-05 ***
Residuals	8	0.00064	0.00008		

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

Repeated Measures ANOVA with PROC GLM: Multivariate Layout

Multivariate Layout

<i>id</i>	<i>RQ_standard</i>	<i>RQ_highfat</i>	<i>RQ_lowfat</i>
1	0.80	0.77	0.83
2	0.78	0.77	0.82
3	0.82	0.80	0.87
4	0.76	0.73	0.79
5	0.83	0.83	0.86

```

DATA RQ;
INPUT id RQ_standard RQ_hifat RQ_lowfat;
DATALINES;
1 0.80 0.77 0.83
2 0.78 0.77 0.82
3 0.82 0.80 0.87
4 0.76 0.73 0.79
5 0.83 0.83 0.86
;

PROC GLM DATA=rq;
  MODEL RQ_standard RQ_hifat RQ_lowfat = /NOUNI; %No univariate tests and output
  REPEATED diet 3;
RUN;

```

Repeated Measures ANOVA with PROC GLM: Multivariate Layout

The GLM Procedure

Number of Observations Read 5
Number of Observations Used 5

The GLM Procedure

Repeated Measures Analysis of Variance

Repeated Measures Level Information

Dependent Variable	standard	hifat	lowfat
Level of diet	1	2	3

```
PROC GLM DATA=rq;
  MODEL RQ_standard RQ_hifat RQ_lowfat =;
  REPEATED diet 3;
RUN;
```

Partial Correlation Coefficients from the Error SSCP Matrix / Prob > |r|

DF = 4	standard	hifat	lowfat
standard	1.000000	0.956650 0.0108	0.962981 0.0085
hifat	0.956650 0.0108	1.000000	0.916031 0.0288
lowfat	0.962981 0.0085	0.916031 0.0288	1.000000

Sphericity:
assumes these
correlations are
equal.

Sphericity Tests

Variables	DF	Mauchly's Criterion	Chi-Square	Pr > ChiSq
Transformed Variates	2	0.5619147	1.7292158	0.4212
Orthogonal Components	2	0.703125	1.0566618	0.5896

Fail to reject.
Assume sphericity.

GLM Repeated Measures Results

→ The GLM Procedure
Repeated Measures Analysis of Variance
 Univariate Tests of Hypotheses for Within Subject Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F
diet	2	0.00756000	0.00378000	47.25	<.0001
Error(diet)	8	0.00064000	0.00008000		

RQ differs significantly across the three diets.

Corrected for sphericity

Source	Adj G - G	Pr > F H - F
diet	0.0002	<.0001
Error(diet)		

Estimates used to correct d.f.

Greenhouse-Geisser Epsilon	0.7711
Huynh-Feldt Epsilon	1.1618

For G-G correction: $df(\text{diet}): 2 \times 0.7711 = 1.542$
 $df(\text{error}): 8 \times 0.7711 = 6.1688$

For H-F Correction: $df(\text{diet}): 2 \times 1 = 2$
 $df(\text{error}): 8 \times 1 = 8$

We do not increase or d.f. using these corrections. If the correction value is greater than 1 (e.g., 1.1618 for H-F), we use 1 and do not adjust our d.f.

NOTE that the other variance sources (e.g., the Between Subject SS) are not included by SAS in the multivariate layout.

Repeated Measures ANOVA with R 'car' package: Multivariate Layout

```
library(car)

mod3 <- lm(as.matrix(respquot[, -1]) ~ 1) # need data in "wide" form
diet <- factor(colnames(respquot)[-1])
results <- Anova(mod3, idata=data.frame(diet), idesign=~diet, type="III")
summary(results, multivariate=FALSE)

## Warning in summary.Anova.mlm(results, multivariate = FALSE): HF eps > 1
## treated as 1

##
## Univariate Type III Repeated-Measures ANOVA Assuming Sphericity
##
##              SS num Df Error SS den Df      F      Pr(>F)
## (Intercept) 9.6962      1  0.01236      4 3137.94 6.081e-07 ***
## diet         0.0076      2  0.00064      8   47.25 3.711e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
## Mauchly Tests for Sphericity
##
##      Test statistic p-value
## diet      0.70313 0.58959
##
##
## Greenhouse-Geisser and Huynh-Feldt Corrections
## for Departure from Sphericity
##
##      GG eps Pr(>F[GG])
## diet 0.77108 0.0002439 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##      HF eps      Pr(>F[HF])
## diet 1.161765 3.710774e-05
```

Repeated Measures ANOVA with PROC GLM: Univariate Layout

Univariate Layout

```
DATA RQ2;
INPUT id diet rq;
DATALINES;
1 1 .80
1 2 .77
1 3 .83
2 1 .78
2 2 .77
2 3 .82
3 1 .82
3 2 .80
3 3 .87
4 1 .76
4 2 .73
4 3 .79
5 1 .83
5 2 .83
5 3 .86
;

PROC GLM DATA=rq2;
  CLASS id diet;
  MODEL rq = diet id;
  RANDOM id /TEST;
RUN;
```

Univariate Layout

<i>id</i>	<i>Diet</i>	<i>RQ</i>
1	1	0.80
1	2	0.77
1	3	0.83
2	1	0.78
2	2	0.77
2	3	0.82
3	1	0.82
3	2	0.80
3	3	0.87
4	1	0.76
4	2	0.73
4	3	0.79
5	1	0.83
5	2	0.83
5	3	0.86

Repeated Measures ANOVA: Univariate Layout

The GLM Procedure

Source	Type III Expected Mean Square
diet	$\text{Var}(\text{Error}) + Q(\text{diet})$
id	$\text{Var}(\text{Error}) + 3 \text{Var}(\text{id})$

The GLM Procedure

Tests of Hypotheses for Mixed Model Analysis of Variance

Dependent Variable: rq

Source	DF	Type III SS	Mean Square	F Value	Pr > F
diet	2	0.007560	0.003780	47.25	<.0001
id	4	0.012360	0.003090	38.63	<.0001
Error: MS(Error)	8	0.000640	0.000080000		

RQ differs significantly across the three diets.

```
mod4 <- aov(rq ~ diet + Error(as.factor(subject)), data=respquot.long)
summary(mod4)
```

```
##
## Error: as.factor(subject)
##      Df Sum Sq Mean Sq F value Pr(>F)
## Residuals  4 0.01236 0.00309
##
## Error: Within
##      Df Sum Sq Mean Sq F value Pr(>F)
## diet    2 0.00756 0.00378  47.25 3.71e-05 ***
## Residuals  8 0.00064 0.00008
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Review Repeated Measures Data

Repeated Measures Data:

- 1) The same outcome/response is measured on the same individual under multiple conditions or at multiple times
 - 2) The same outcome/response is measured for clusters or groups of similar individuals
- Two sample t-test is to a paired t-test as an ANOVA is to a Repeated Measures ANOVA

Assumptions:

- 1) Normality: Each population is normally distributed (errors follow a normal distribution)
 - 2) Sphericity: homogeneity of variances and of covariances
 - 3) Independence of experimental units: Individuals or clusters are independent
- If sphericity is violated, then adjust the degrees of freedom or use **Mixed Models** approach

Note: two-sample t tests, paired t tests, ANOVA, Repeated Measures ANOVA do not easily account for confounders (Stratify but then reduce sample size)