stcp-karadimitriou-ANOVArepeatedR

The following resources are associated: The dataset *'Cholesterol.csv'* and Repeated measures ANOVA in R script file. Checking normality in R, Paired t-test in R and Friedman in R resources.

# Repeated measures (within-subjects) ANOVA in R

**Dependent variable:** Continuous (scale)

**Independent variable**: Categorical e.g. time/ condition (within subjects factor)

**Common Applications:** Used when several measurements of the same dependent variable are taken at different time points or under different conditions. Repeated measures ANOVA analyses (1) changes in mean score over 3 or more time points or (2) differences in mean score under 3 or more conditions. This is the equivalent of a one-way ANOVA but for repeated samples and is an extension of a paired-samples t-test. Repeated measures ANOVA is also known as 'within-subjects' ANOVA.

**Data:** Participants used Clora margarine for 8 weeks. Their cholesterol (in mmol/L) was measured before the special diet, after 4 weeks and after 8 weeks. Open the csv file '*Cholesterol.csv*' and call it cholA, changing the command depending on where you have saved the file and what you called it, then use `attach (cholA)` so that the variable names can be used in future calculations.

```
cholA<-read.csv("D:\\cholesterol.csv",header=T)
attach(cholA)
```

| Subject | Before | After4weeks | After8weeks | Margarine |
|---|---|---|---|---|
| 1 | 6.42 | 5.83 | 5.75 | B |
| 2 | 6.76 | 6.20 | 6.13 | A |
| 3 | 6.56 | 5.83 | 5.71 | B |
| 4 | 4.80 | 4.27 | 4.15 | A |

There is one row person with their cholesterol at the three time points in different columns (Before, After 4 weeks and After 8 weeks). e.g. The 'After4weeks' column contains the cholesterol measurements after 4 weeks on the diet.
Note: Ignore the 'Margarine' column for now.

### Assumptions for repeated measures ANOVA

| Assumptions | How to check | What if the assumption is not met |
|---|---|---|
| The data at each time point are approximately normally distributed. | Use histograms/ normality tests to check the dependent is approximately normally distributed by group. | If the data are **very** skewed, ANOVA is not reliable so use the non-parametric Friedman test instead (See *Friedman in R* resource) |
| Sphericity: the variances of the differences between all combinations of the related conditions/ time points are equal (similar to the assumption of equal variances in ANOVA). | *Mauchly's test of Sphericity* is automatically given in the output. If $p > 0.05$, Sphericity can be assumed. | Use the p-value from the Greenhouse-Geisser correction from the *Sphericity Corrections* output |

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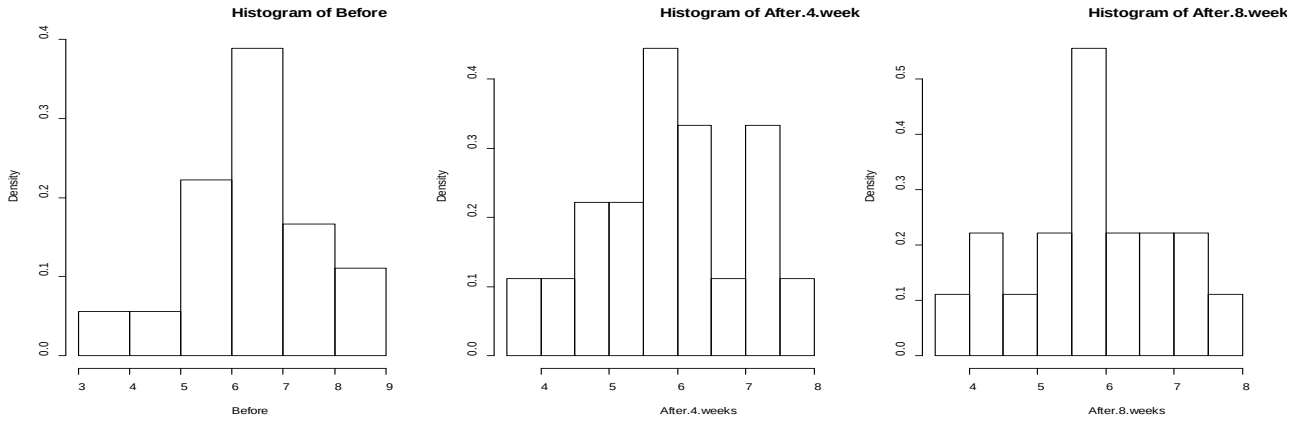
**Checking normality**

To check the normality of the dependent variable at each time point, use the dataset cholA which has one row per person and separate columns for each time point.

Check the normality of the cholesterol measurements by time by producing histograms.

```
par(mfrow=c(1,3))
hist(Before,prob=T)
hist(After4weeks, prob=T)
hist(After8weeks,prob=T)
```




**Preparing the data for analysis**

The dataset with one row per person (wide format) is suitable for carrying out the post hoc tests and checking the assumption of normality but for the actual repeated measures ANOVA, it will have to be reformatted so that there are three rows per person, one row for each time point (long format). This means that we need the cholesterol measurements to be in one variable, another variable will specify the time point when the measurement was taken (1, 2 or 3) and a third will identify the person (1 – 18).

Tell R you want a new dataset called cholB which has 53 rows of data and 3 columns using `nrow()` and `ncol()`. Replicate the subject ID for the 18 participants 3 times using `rep(1:18,3)`. Combine the measurements for each time point into one column. Create a column to identify which time point each observation came from using `rep(1,18)`, `rep(2,18)` etc. Replicate the margarine type for the 18 participants 3 times using `rep(cholA$Margarine,3)`.

```
cholB<-matrix(nrow=54,ncol=4,c(rep(1:18,3),cholA$Before,
cholA$After4weeks,cholA$After8weeks,rep(1,18),rep(2,18),rep(3,18),rep(cho
lA$Margarine,3)))
```

Tell R cholB is a dataset and give each column a label using *colnames*.

```
cholB<-data.frame(cholB)
colnames(cholB)<-c('subject','cholesterol','time','Margarine')
```

Tell R that time, subject and Margarine are categorical variables using factor(variable) e.g.

```
cholB$time<-factor(cholB$time)
```

Tell R to use the dataset using `attach(cholB)` e.g. 'time' can be used instead of cholB$time.

Look at the dataset to see if the above commands have worked.

```
cholB
```

| | subject | cholesterol | time |
|---|---|---|---|
| 1 | 1 | 6.42 | 1 |
| 2 | 2 | 6.76 | 1 |
| 3 | 3 | 6.56 | 1 |

CholB is now formatted for repeated measures ANOVA with a column identifying the person, a column for the dependent cholesterol and a column identifying the independent variable time.

**Steps in R**

There are three steps when carrying out a repeated measures ANOVA:

1. Check the assumptions
2. The ANOVA reports whether there are any differences between time points
3. If significant, carry out post hoc tests to compare pairs of means

In order to fit the repeated measures ANOVA the library ez needs to be loaded. In R, go to Packages (Tools) → Install packages and choose ez. This package will then download to your temporary drive. (Note: Earlier versions of Rstudio may not run the commands so download the latest version or use the original R interface). Then use `library(ez)` to load the package and enable use of ez.

Load the library

```
library(ez)
```

Fit the repeated measures ANOVA using the command as `ezANOVA()` and ask to view the output For this example, specify the dependent variable (`dv`) as the cholesterol level, the subject identifier as subject (`wid`), and the within-subject factor (`within`) as time.

```
repeat1<ezANOVA(data=cholB,dv=.(cholesterol),wid=.(subject),within=.(time
),type=3)
repeat1
$ANOVA
  Effect DFn DFd        F            p p<.05        ges
2   time   2  34 212.3206 6.167367e-20     * 0.06124278

$`Mauchly's Test for Sphericity`
  Effect         W            p p<.05
2   time 0.3809941 0.0004439621     *

$`Sphericity Corrections`
  Effect      GGe        p[GG] p[GG]<.05       HFe        p[HF] p[HF]<.05
2   time 0.617663 3.887911e-13         * 0.6418105 1.442841e-13         *
```

If p < 0.05, the assumption of sphericity has not been met so use the Sphericity Corrections table instead of the ANOVA table

The first thing to look at is the Mauchly's test of sphericity to decide whether to use the ANOVA table or the Sphericity Corrections table. If p < 0.05, use the sphericity corrections table as the assumption for the repeated measures has not been met. **If the assumption of sphericity is met**, report from the first ANOVA table [F(2, 34)= 212.32, p < 0.001].

Here the assumption of sphericity has not been met (p = 0.00044) so the 'Sphericity corrections' table. There are two tests in the Sphericity corrections table, the Greenhouse-Geisser (GG) and Huynh-Feldt (HF) which both make adjustments to the degrees of freedom (DFn and DFd) from the repeated measures ANOVA. The degrees of freedom for time (DFn=$df_{time}$) are calculated as the number of time points – 1.

The GGe value is known as epsilon. The epsilon measures how far the data is from the ideal sphericity and ranges between 0 and 1 where 1 is no departure from sphericity. If the epsilon of Greenhouse-Geisser is greater than 0.75 or there is a small sample size (e.g. 10), the epsilon of Huynh-Feldt should be used. This is because Greenhouse-Geisser tends to make the analysis too strict when the epsilon is large. As the GGe value is less than 0.75, use the Greenhouse-Geisser adjustment of 0.618. The p value with the adjusted F value is available next to the epsilon (p[GG]). In this example, as p<0.001 there is evidence of a difference between at least two time points

For reporting the results use F($df_{time}$, $df_{error}$)= Test statistic [F, p = …].

If the Greenhouse-Geisser correction is used, make the correction to the degrees of freedom by multiply the degrees of freedom (DFn and DFd) by the GGe value e.g 2 * 0.618 = 1.236. There

was significant evidence, F(1.235, 21.01)= 212.32, p < 0.001, to suggest a difference between at least two time points.

**Post hoc tests** (**Pairwise Comparisons)**

R does not carry out pairwise comparisons for repeated measures so conduct pairwise paired t-tests between the weeks and then do a Bonferroni adjustment of the p-values. The Bonferroni adjustment multiplies the p-value for each test by the number of tests being carried out. For example, here the t-tests are time points 1 vs 2, 1 vs 3 and 2 vs 3 so there are 3 tests and each test p-value is multiplied by 3. Paired t-tests require that the values for each time point are in separate columns (the original format of this data). Refer to the *Paired t-test in R* resource for more details. Tell R to use the original data cholA again.

```
attach(cholA)
```

Carry out pairwise paired t-tests t-test and save them as objects. T1 is the t-test to look at the change in cholesterol from the start to the 4 week measurement.

```
t1<-t.test(Before,After4weeks,paired=T)
t2<-t.test(Before,After8weeks,paired=T)
t3<-t.test(After4weeks,After8weeks,paired=T)
```

Report the pvalues of each test in one row.

```
pvalues<-c(t1$p.value,t2$p.value,t3$p.value)
```

Adjust the p-values using the bonferroni correction for 3 tests (rounded to 4 decimals).

```
round(p.adjust(pvalues,'bonferroni',3),4)
[1] 0.0000 0.0000 0.0045
```

There is significant evidence of a difference between all time points.

Ask to view the output for each e.g `t1` and produce a table to summarise the output

| Test | Mean difference | Test Statistic | Adjusted p-value |
|---|---|---|---|
| Before - 4 weeks | 0.566 | 15.44 | p < 0.001 |
| Before - 8 weeks | 0.63 | 14.95 | p < 0.001 |
| 4 weeks – 8 weeks | 0.063 | 3.78 | p = 0.0045 |

There was a significant difference between each pair of time points. Cholesterol reduced by 0.566 mmol/L between baseline and 4 weeks (p < 0.001) and then reduced by an additional 0.063 mmol/L between 4 and 8 weeks (p = 0.0045).

**Reporting ANOVA**

Participants used Clora margarine for 8 weeks. Their cholesterol was measured before the special diet, after 4 weeks and after 8 weeks. Normality checks were carried out on the dependent variable by group, which were approximately normally distributed. A repeated measures ANOVA with a Greenhouse-Geisser correction showed that mean cholesterol differed significantly between time points [F(1.235, 21.01)= 212.32, p < 0.001]. Post hoc tests using the Bonferroni correction revealed that Cholesterol reduced by an average of 0.566 mmol/L after 4 weeks (p < 0.001) and then reduced by an additional 0.063 mmol/L between 4 and 8 weeks (p = 0.0045).

Note: Does the change in mean cholesterol look meaningful?

To assess this, look at the starting mean. Cholesterol drops by approximately 9% after 4 weeks which is meaningful but only drops by approximately 1% between 4 and 8 weeks which seems less meaningful. Always check significant results to see if the change is meaningful. A large sample size or small standard deviations result in significance for small differences.