Mixed ANOVA in R

Mixed ANOVA is used to compare the means of groups cross-classified by two different types of factor variables, including:

- **between-subjects factors**, which have independent categories (e.g., gender: male/female)
- **within-subjects factors**, which have related categories also known as repeated measures (e.g., time: before/after treatment).

The mixed ANOVA test is also referred as mixed design ANOVA and mixed measures ANOVA.

This chapter describes different types of mixed ANOVA, including:

- two-way mixed ANOVA, used to compare the means of groups cross-classified by two
 independent categorical variables, including one between-subjects and one within-subjects
 factors.
- three-way mixed ANOVA
 - , used to evaluate if there is a three-way interaction between three independent variables, including between-subjects and within-subjects factors. You can have two different designs for three-way mixed ANOVA:
 - 1. one between-subjects factor and two within-subjects factors
 - 2. two between-subjects factor and one within-subjects factor

You will learn how to:

- Compute and interpret the different mixed ANOVA tests in R.
- Check mixed ANOVA test assumptions
- **Perform post-hoc tests**, multiple pairwise comparisons between groups to identify which groups are different
- Visualize the data using box plots, add ANOVA and pairwise comparisons p-values to the plot

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Related Book

Practical Statistics in R II - Comparing Groups: Numerical Variables

Assumptions

The mixed ANOVA makes the following assumptions about the data:

- **No significant outliers** in any cell of the design. This can be checked by visualizing the data using box plot methods and by using the function <code>identify_outliers()</code> [rstatix package].
- Normality: the outcome (or dependent) variable should be approximately normally distributed in each cell of the design. This can be checked using the Shapiro-Wilk normality test (shapiro_test() [rstatix]) or by visual inspection using QQ plot (ggqqplot() [ggpubr package]).
- **Homogeneity of variances**: the variance of the outcome variable should be equal between the groups of the between-subjects factors. This can be assessed using the **Levene's test for equality of variances** ([levene_test()] [rstatix]).
- **Assumption of sphericity**: the variance of the differences between within-subjects groups should be equal. This can be checked using the **Mauchly's test of sphericity**, which is automatically reported when using the anova_test() R function.
- **Homogeneity of covariances** tested by Box's M. The covariance matrices should be equal across the cells formed by the between-subjects factors.

Before computing mixed ANOVA test, you need to perform some preliminary tests to check if the assumptions are met.

Prerequisites

Make sure that you have installed the following R packages:

- tidyverse for data manipulation and visualization
- ggpubr for creating easily publication ready plots
- rstatix provides pipe-friendly R functions for easy statistical analyses
- datarium: contains required data sets for this chapter

Start by loading the following R packages:

```
1 library(tidyverse)
2 library(ggpubr)
3 library(rstatix)
```

Key R functions:

```
• 1 anova_test()
```

[rstatix package], a wrapper around

```
1 | car::Anova()
```

for making easy the computation of repeated measures ANOVA. Key arguments for performing repeated measures ANOVA:

- o data: data frame
- o dv: (numeric) the dependent (or outcome) variable name.
- wid: variable name specifying the case/sample identifier.
- between: between-subjects factor or grouping variable
- within: within-subjects factor or grouping variable
- get_anova_table() [rstatix package]. Extracts the ANOVA table from the output of anova_test(). It returns ANOVA table that is automatically corrected for eventual deviation from the sphericity assumption. The default is to apply automatically the Greenhouse-Geisser sphericity correction to only within-subject factors violating the sphericity assumption (i.e., Mauchly's test p-value is significant, p <= 0.05). Read more in Chapter @ref(mauchly-s-test-of-sphericity-in-r).</p>

Two-way mixed ANOVA

Data preparation

We'll use the anxiety dataset [in the datarium package], which contains the anxiety score, measured at three time points (t1, t2 and t3), of three groups of individuals practicing physical exercises at different levels (grp1: basal, grp2: moderate and grp3: high)

Two-way mixed ANOVA can be used to evaluate if there is interaction between group and time in explaining the anxiety score.

Load and show one random row by group:

```
1  # wide format
2  set.seed(123)
3  data("anxiety", package = "datarium")
4  anxiety %>% sample_n_by(group, size = 1)
5  ## # A tibble: 3 x 5
6  ## id group t1 t2 t3
7  ## <fct> <fct> <dbl> <dbl> <dbl> <dbl> 8
## 1 5  grp1 16.5 15.8 15.7
```

```
9 ## 2 27 grp2 17.8 17.7 16.9
10 ## 3 37
             grp3 17.1 15.6 14.3
11 # Gather the columns t1, t2 and t3 into long format.
12 # Convert id and time into factor variables
   anxiety <- anxiety %>%
13
14
     gather(key = "time", value = "score", t1, t2, t3) %>%
     convert_as_factor(id, time)
15
16 # Inspect some random rows of the data by groups
    set.seed(123)
17
    anxiety %>% sample_n_by(group, time, size = 1)
18
19 | ## # A tibble: 9 x 4
20 ## id
           group time score
21 ## <fct> <fct> <fct> <dbl>
22 ## 1 5
           grp1 t1
                      16.5
23 ## 2 12 grp1 t2
                        17.7
24 ## 3 7
            grp1 t3
                       16.5
25 ## 4 29 grp2 t1
                       18.4
26 ## 5 30 grp2 t2
                       18.9
           grp2 t3
27 ## 6 16
                        12.7
```

Summary statistics

Group the data by time and group, and then compute some summary statistics of the score variable: mean and sd (standard deviation)

```
1 anxiety %>%
    group_by(time, group) %>%
3
    get_summary_stats(score, type = "mean_sd")
4 ## # A tibble: 9 x 6
5 ## group time variable
                           n mean
6 ##
      <fct> <fct> <chr> <dbl> <dbl> <dbl> <dbl>
7
   ## 1 grp1 t1 score
                          15 17.1 1.63
   ## 2 grp2 t1 score
                           15 16.6 1.57
   ## 3 grp3 t1 score
                           15 17.0 1.32
10
   ## 4 grp1 t2
                score
                           15 16.9 1.70
11 ## 5 grp2 t2 score
                           15 16.5 1.70
12 | ## 6 grp3 t2 score 15 15.0 1.39
13 | ## # ... with 3 more rows
```

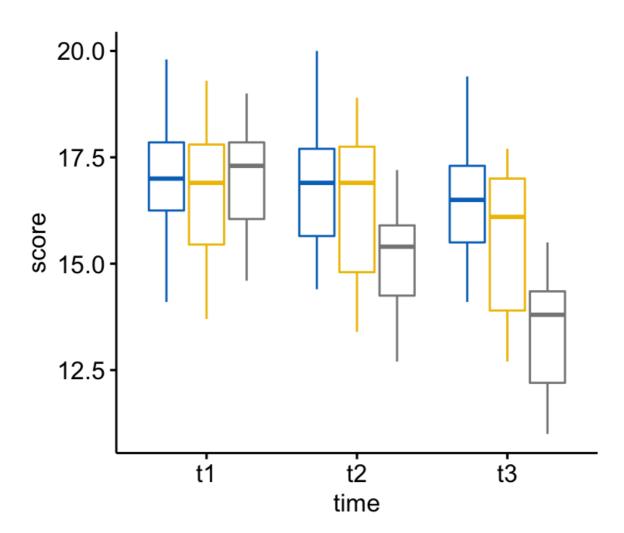
Visualization

Create a box plots:

```
bxp <- ggboxplot(
anxiety, x = "time", y = "score",
color = "group", palette = "jco"

bxp</pre>
```





Check assumptions

Outliers

Outliers can be easily identified using box plot methods, implemented in the R function identify_outliers() [rstatix package].

```
1 anxiety %>%
2  group_by(time, group) %>%
3  identify_outliers(score)
4  ## [1] group time id score is.outlier is.extreme
5  ## <0 rows> (or 0-length row.names)
```

There were no extreme outliers.

Note that, in the situation where you have extreme outliers, this can be due to: 1) data entry errors, measurement errors or unusual values.

Yo can include the outlier in the analysis anyway if you do not believe the result will be substantially affected. This can be evaluated by comparing the result of the ANOVA with and without the outlier.

It's also possible to keep the outliers in the data and perform robust ANOVA test using the WRS2 package.

Normality assumption

The normality assumption can be checked by computing Shapiro-Wilk test for each combinations of factor levels. If the data is normally distributed, the p-value should be greater than 0.05.

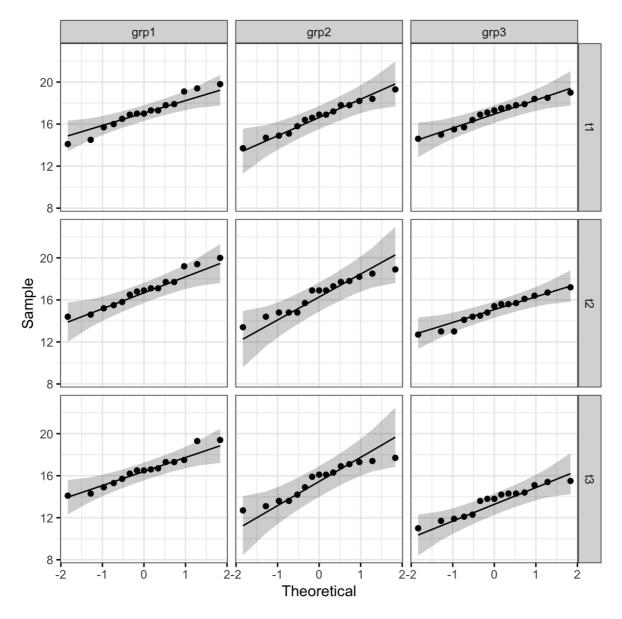
```
1 anxiety %>%
 2
    group_by(time, group) %>%
     shapiro_test(score)
 4 ## # A tibble: 9 x 5
 5 | ## group time variable statistic
 6 | ## <fct> <fct> <chr> <dbl> <dbl>
 7 ## 1 grp1 t1 score
                              0.964 0.769
                             0.977 0.949
0.954 0.588
8 ## 2 grp2 t1 score
9 ## 3 grp3 t1 score
10 | ## 4 grp1 t2 score
                             0.956 0.624
11 ## 5 grp2 t2 score
                              0.935 0.328
12 ## 6 grp3 t2 score 0.952 0.558
13 | ## # ... with 3 more rows
```

The score were normally distributed (p > 0.05) for each cell, as assessed by Shapiro-Wilk's test of normality.

Note that, if your sample size is greater than 50, the normal QQ plot is preferred because at larger sample sizes the Shapiro-Wilk test becomes very sensitive even to a minor deviation from normality.

QQ plot draws the correlation between a given data and the normal distribution.

```
1 ggqqplot(anxiety, "score", ggtheme = theme_bw()) +
2 facet_grid(time ~ group)
```



All the points fall approximately along the reference line, for each cell. So we can assume normality of the data.

In the situation where the assumptions are not met, you could consider running the two-way repeated measures ANOVA on the transformed or performing a robust ANOVA test using the WRS2 R package.

Homogneity of variance assumption

The homogeneity of variance assumption of the between-subject factor (group) can be checked using the Levene's test. The test is performed at each level of time variable:

```
1
  anxiety %>%
2
    group_by(time) %>%
3
   levene_test(score ~ group)
  ## # A tibble: 3 x 5
4
5
       time
               df1 df2 statistic
6
       <fct> <int> <int>
                           <dbl> <dbl>
7
               2
                           0.176 0.839
  ## 1 t1
                     42
8
  ## 2 t2
                 2
                      42
                           0.249 0.781
9
   ## 3 t3
                      42
                            0.335 0.717
```

There was homogeneity of variances, as assessed by Levene's test (p > 0.05).

Note that, if you do not have homogeneity of variances, you can try to transform the outcome (dependent) variable to correct for the unequal variances.

It's also possible to perform robust ANOVA test using the WRS2 R package.

Homogeneity of covariances assumption

The homogeneity of covariances of the between-subject factor (group) can be evaluated using the **Box's M-test** implemented in the rstatix package. If this test is statistically significant (i.e., p < 0.001), you do not have equal covariances, but if the test is not statistically significant (i.e., p > 0.001), you have equal covariances and you have not violated the assumption of homogeneity of covariances.

Note that, the Box's M is highly sensitive, so unless p < 0.001 and your sample sizes are unequal, ignore it. However, if significant and you have unequal sample sizes, the test is not robust (https://en.wikiversity.org/wiki/Box%27s M, Tabachnick & Fidell, 2001).

Compute Box's M-test:

There was homogeneity of covariances, as assessed by Box's test of equality of covariance matrices (p > 0.001).

Note that, if you do not have homogeneity of covariances, you could consider separating your analyses into distinct repeated measures ANOVAs for each group. Alternatively, you could omit the interpretation of the interaction term.

Unfortunately, it is difficult to remedy a failure of this assumption. Often, a mixed ANOVA is run anyway and the violation noted.

Assumption of sphericity

As mentioned in previous sections, the assumption of sphericity will be automatically checked during the computation of the ANOVA test using the R function anova_test() [rstatix package]. The Mauchly's test is internally used to assess the sphericity assumption.

By using the function <code>get_anova_table()</code> [rstatix] to extract the ANOVA table, the Greenhouse-Geisser sphericity correction is automatically applied to factors violating the sphericity assumption.

Computation

```
# Two-way mixed ANOVA test
2 res.aov <- anova_test(</pre>
3
   data = anxiety, dv = score, wid = id,
   between = group, within = time
4
5
   )
6
   get_anova_table(res.aov)
7 ## ANOVA Table (type II tests)
8 ##
        Effect DFn DFd F p p<.05 ges
9 ##
10 ## 1
         group 2 42 4.35 1.90e-02 * 0.168
11 ## 2 time 2 84 394.91 1.91e-43 * 0.179
```

From the output above, it can be seen that, there is a statistically significant two-way interactions between group and time on anxiety score, F(4, 84) = 110.18, p < 0.0001.

Post-hoc tests

A **significant two-way interaction** indicates that the impact that one factor has on the outcome variable depends on the level of the other factor (and vice versa). So, you can decompose a significant two-way interaction into:

- **Simple main effect**: run one-way model of the first variable (factor A) at each level of the second variable (factor B),
- **Simple pairwise comparisons**: if the simple main effect is significant, run multiple pairwise comparisons to determine which groups are different.

For a **non-significant two-way interaction**, you need to determine whether you have any statistically significant **main effects** from the ANOVA output.

Procedure for a significant two-way interaction

Simple main effect of group variable. In our example, we'll investigate the effect of the between-subject factor group on anxiety score at every time point.

```
1 # Effect of group at each time point
2 one.way <- anxiety %>%
3
   group_by(time) %>%
    anova_test(dv = score, wid = id, between = group) %>%
4
5
   get_anova_table() %>%
    adjust_pvalue(method = "bonferroni")
6
7 one.way
8 ## # A tibble: 3 x 9
9
  ## time Effect DFn DFd F
                                       p `p<.05` ges p.adj
11 ## 1 t1 group 2 42 0.365 0.696 "" 0.017 1
                   2 42 5.84 0.006
                                       *
12 ## 2 t2
                                             0.218 0.018
            group
13 | ## 3 t3 group
                   2 42 13.8 0.0000248 * 0.396 0.0000744
14 | # Pairwise comparisons between group levels
15 pwc <- anxiety %>%
   group_by(time) %>%
16
17
   pairwise_t_test(score ~ group, p.adjust.method = "bonferroni")
18 pwc
  ## # A tibble: 9 x 10
19
```

```
20
                                             n2
    ## time .y. group1 group2
                                    n1
                                                      p p.signif
                                                                    p.adj
    p.adj.signif
    ## * <fct> <chr> <chr> <chr> <int> <int>
                                                  <db1> <chr>
                                                                    <db1> <chr>
21
    ## 1 t1
                                       15
                                             15 0.43
                                                                  1
22
               score grp1
                             grp2
                                                        ns
                                                                          ns
    ## 2 t1
23
               score grp1
                                       15
                                             15 0.895
                                                                  1
                             grp3
                                                        ns
                                                                          ns
    ## 3 t1
                                             15 0.51
24
               score grp2
                             grp3
                                       15
                                                        ns
                                                                  1
                                                                          ns
    ## 4 t2
               score grp1
                                       15
                                             15 0.435
25
                             grp2
                                                        ns
                                                                  1
                                                                          ns
    ## 5 t2
                                             15 0.00212 **
                                                                  0.00636 **
                                       15
26
               score grp1
                             grp3
    ## 6 t2
                                       15
                                             15 0.0169 *
                                                                  0.0507 ns
27
               score grp2
                             grp3
    ## # ... with 3 more rows
```

Considering the Bonferroni adjusted p-value (p.adj), it can be seen that the simple main effect of group was significant at t2 (p = 0.018) and t3 (p < 0.0001) but not at t1 (p = 1).

Pairwise comparisons show that the mean anxiety score was significantly different in grp1 vs grp3 comparison at t2 (p = 0.0063); in grp1 vs grp3 (p < 0.0001) and in grp2 vs grp3 (p = 0.0013) at t3.

Simple main effects of time variable. It's also possible to perform the same analyze for the within-subject time variable at each level of group as shown in the following R code. You don't necessarily need to do this analysis.

```
# Effect of time at each level of exercises group
    one.way2 <- anxiety %>%
 2
 3
      group_by(group) %>%
      anova_test(dv = score, wid = id, within = time) %>%
 4
 5
      get_anova_table() %>%
 6
      adjust_pvalue(method = "bonferroni")
    one.way2
 7
 8
    ## # A tibble: 3 x 9
 9
         group Effect DFn
                                                p `p<.05`
    ##
                              DFd
                                       F
                                                                   p.adj
                                                            ges
10
         <fct> <chr> <dbl> <dbl> <dbl> <dbl>
                                            <dbl> <chr>
                                                          <dbl>
                                                                   <db1>
    ## 1 grp1 time
                          2
                               28 14.8 4.05e- 5 *
                                                          0.024 1.21e- 4
11
                          2
12
    ## 2 grp2 time
                               28 77.5 3.88e-12 *
                                                          0.086 1.16e-11
                          2
                               28 490. 1.64e-22 *
13
    ## 3 grp3 time
                                                          0.531 4.92e-22
14
    # Pairwise comparisons between time points at each group levels
    # Paired t-test is used because we have repeated measures by time
15
16
    pwc2 <- anxiety %>%
17
      group_by(group) %>%
      pairwise_t_test(
18
19
        score ~ time, paired = TRUE,
20
        p.adjust.method = "bonferroni"
21
        ) %>%
22
      select(-df, -statistic, -p) # Remove details
23
    pwc2
24
    ## # A tibble: 9 x 8
         group .y.
                                                       p.adj p.adj.signif
25
                    group1 group2
                                             n2
                                       n1
    ## * <fct> <chr> <chr> <chr> <int> <int>
                                                       <db1> <chr>
26
    ## 1 grp1 score t1
                                      15
                                             15 0.194
27
                            t2
                                                             ns
```

```
28 ## 2 grp1 score t1 t3 15 0.002 **

29 ## 3 grp1 score t2 t3 15 0.006 **

30 ## 4 grp2 score t1 t2 15 0.268 ns

31 ## 5 grp2 score t1 t3 15 0.000000151 ***

32 ## 6 grp2 score t2 t3 15 0.0000000612 ***

33 ## # ... with 3 more rows
```

There was a statistically significant effect of time on anxiety score for each of the three groups. Using pairwise paired t-test comparisons, it can be seen that for grp1 and grp2, the mean anxiety score was not statistically significantly different between t1 and t2 time points.

The pairwise comparisons t1 vs t3 and t2 vs t3 were statistically significantly different for all groups.

Procedure for non-significant two-way interaction

If the interaction is not significant, you need to interpret the main effects for each of the two variables: group and `time. A significant main effect can be followed up with pairwise comparisons.

In our example, there was a statistically significant main effects of group (F(2, 42) = 4.35, p = 0.02) and time (F(2, 84) = 394.91, p < 0.0001) on the anxiety score.

Perform multiple pairwise paired t-tests for the time variable, ignoring group. P-values are adjusted using the Bonferroni multiple testing correction method.

```
1 anxiety %>%
2 pairwise_t_test(
3 score ~ time, paired = TRUE,
4 p.adjust.method = "bonferroni"
5 )
```

All pairwise comparisons are significant.

You can perform a similar analysis for the group variable.

```
1 anxiety %>%
2 pairwise_t_test(
3 score ~ group,
4 p.adjust.method = "bonferroni"
5 )
```

All pairwise comparisons are significant except grp1 vs grp2.

Report

There was a statistically significant interaction between exercises group and time in explaining the anxiety score, F(4, 84) = 110.19, p < 0.0001.

Considering the Bonferroni adjusted p-value, the simple main effect of exercises group was significant at t2 (p = 0.018) and t3 (p < 0.0001) but not at t1 (p = 1).

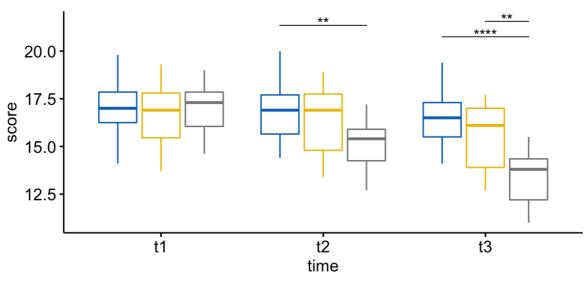
Pairwise comparisons show that the mean anxiety score was significantly different in grp1 vs grp3 comparison at t2 (p = 0.0063); in grp1 vs grp3 (p < 0.0001) and in grp2 vs grp3 (p = 0.0013) at t3.

Note that, for the plot below, we only need the pairwise comparison results for t2 and t3 but not for t1 (because the simple main effect of exercises group was not significant at this time point). We'll filter the comparison results accordingly.

```
# Visualization: boxplots with p-values
1
2
   pwc <- pwc %>% add_xy_position(x = "time")
3
   pwc.filtered <- pwc %>% filter(time != "t1")
4
   bxp +
5
     stat_pvalue_manual(pwc.filtered, tip.length = 0, hide.ns = TRUE) +
6
7
       subtitle = get_test_label(res.aov, detailed = TRUE),
8
       caption = get_pwc_label(pwc)
9
     )
```

Anova,
$$F(4.84) = 110.19$$
, $p = <0.0001$, $\eta_g^2 = 0.11$





pwc: T test; p.adjust: Bonferroni

Three-way mixed ANOVA: 2 between- and 1 withinsubjects factors

This section describes how to compute the three-way mixed ANOVA, in R, for a situation where you have **two between-subjects factors and one within-subjects factor**.

This setting is for investigating group differences over time (i.e., the within-subjects factor) where groups are formed by the combination of two between-subjects factors. For example, you might want to understand how performance score changes over time (e.g., 0, 4 and 8 months) depending on gender (i.e., male/female) and stress (low, moderate and high stress).

Data preparation

We'll use performance dataset [datarium package] containing the performance score measures of participants at two time points. The aim of this study is to evaluate the effect of gender and stress on performance score.

The data contains the following variables:

- 1. Performance score (outcome or dependent variable) measured at two time points, t1 and t2.
- 2. Two between-subjects factors: gender (levels: male and female) and stress (low, moderate, high)
- 1. One within-subjects factor, time, which has two time points: t1 and t2.

Load and inspect the data by showing one random row by group:

```
# Load and inspect the data
2 # Wide format
3 set.seed(123)
4 data("performance", package = "datarium")
5 performance %>% sample_n_by(gender, stress, size = 1)
6 ## # A tibble: 6 x 5
7
   ##
         id gender stress
                           t1 t2
   ## <int> <fct> <fct> <dbl> <dbl>
8
9
   ## 1 3 male low
                          5.63 5.47
10 ## 2 18 male moderate 5.57 5.78
11 ## 3 25 male high 5.48 5.74
12 ## 4 39 female low 5.50 5.66
13 ## 5 50 female moderate 5.96 5.32
14 ## 6 51 female high 5.59 5.06
15 # Gather the columns t1, t2 and t3 into long format.
16 | # Convert id and time into factor variables
17 performance <- performance %>%
    gather(key = "time", value = "score", t1, t2) %>%
18
19
     convert_as_factor(id, time)
20 # Inspect some random rows of the data by groups
21 set.seed(123)
22 performance %>% sample_n_by(gender, stress, time, size = 1)
23 | ## # A tibble: 12 x 5
24 ## id gender stress time score
25 ## <fct> <fct> <fct> <fct> <dbl>
26 ## 1 3 male low
                          t1 5.63
27 ## 2 8 male low t2
                                5.92
28 ## 3 15 male moderate t1 5.96
29 | ## 4 19 male moderate t2
                               5.76
30 ## 5 30 male high t1
                                5.38
31 ## 6 21 male high
                         t2
                                5.64
32 | ## # ... with 6 more rows
```

Summary statistics

Group the data by gender, stress and time, and then compute some summary statistics of the score variable: mean and sd (standard deviation)

```
performance %>%
     group_by(gender, stress, time ) %>%
 3
     get_summary_stats(score, type = "mean_sd")
   ## # A tibble: 12 x 7
        gender stress time variable
                                       n mean
 6
        <fct> <fct>
                      <fct> <chr>
                                   <fdb> <fdb> <fdb>
7
   ## 1 male low
                      t1 score
                                     10 5.72 0.19
                      t2
   ## 2 male
                                      10 5.70 0.143
              low
                            score
   ## 3 male moderate t1 score
9
                                     10 5.72 0.193
10
   ## 4 male moderate t2
                           score
                                      10 5.77 0.155
   ## 5 male high
                                     10 5.48 0.121
11
                     t1 score
                           score
   ## 6 male high
                                      10 5.64 0.195
12
   ## # ... with 6 more rows
13
```

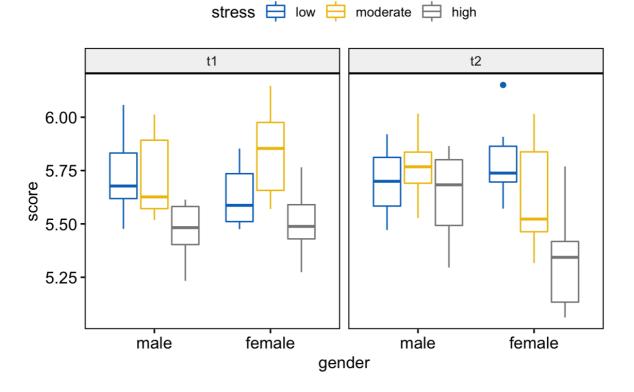
Visualization

Create box plots of performance score by gender colored by stress levels and faceted by time:

```
bxp <- ggboxplot(
performance, x = "gender", y = "score",
color = "stress", palette = "jco",
facet.by = "time"

bxp

bxp</pre>
```



Check assumptions

Outliers

```
performance %>%
group_by(gender, stress, time) %>%
identify_outliers(score)

## # A tibble: 1 x 7

## gender stress time id score is.outlier is.extreme
## <fct> <fct> <fct> <fct> <fct> <dbl> <lgl> <lgl>
## 1 female low t2 36 6.15 TRUE FALSE
```

There were no extreme outliers.

Normality assumption

Compute Shapiro-Wilk test for each combinations of factor levels:

```
performance %>%
group_by(gender, stress, time ) %>%
shapiro_test(score)

## # A tibble: 12 x 6

## gender stress time variable statistic p
## cfct> <fct> <chr> <dbl> <dbl> <dbl> <dbl> <dbl> </d>

## 1 male low t1 score 0.942 0.574

## 2 male low t2 score 0.966 0.849

## 3 male moderate t1 score 0.848 0.0547

## 4 male moderate t2 score 0.958 0.761

## 5 male high t1 score 0.915 0.319

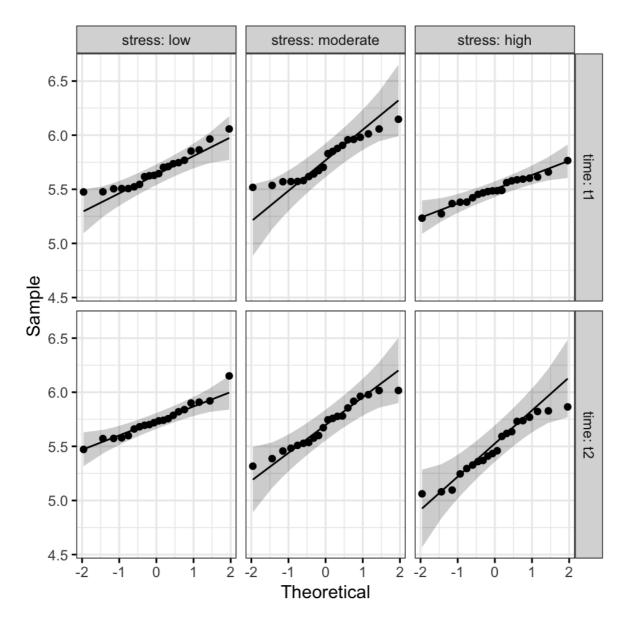
## 6 male high t2 score 0.925 0.403

## # ... with 6 more rows
```

The score were normally distributed (p > 0.05) for each cell, as assessed by Shapiro-Wilk's test of normality.

Create QQ plot for each cell of design:

```
ggqqplot(performance, "score", ggtheme = theme_bw()) +
facet_grid(time ~ stress, labeller = "label_both")
```



All the points fall approximately along the reference line, for each cell. So we can assume normality of the data.

Homogneity of variance assumption

Compute the Levene's test at each level of the within-subjects factor, here time variable:

```
performance %>%
2
    group_by(time) %>%
    levene_test(score ~ gender*stress)
3
  ## # A tibble: 2 x 5
5
       time
             df1 df2 statistic
       <fct> <int> <int>
                           <dbl> <dbl>
6
                5
                     54
                            0.974 0.442
  ## 1 t1
  ## 2 t2
                 5
                     54
                           0.722 0.610
```

There was homogeneity of variances, as assessed by Levene's test of homogeneity of variance (p > .05).

Assumption of sphericity

As mentioned in the two-way mixed ANOVA section, the Mauchly's test of sphericity and the sphericity corrections are internally done using the R function anova_test() and get_anova_table() [rstatix package].

Computation

```
1 res.aov <- anova_test(</pre>
 2
     data = performance, dv = score, wid = id,
     within = time, between = c(gender, stress)
 4
      )
 5 get_anova_table(res.aov)
 6 | ## ANOVA Table (type II tests)
 7 ##
 8 ##
                     Effect DFn DFd F p p<.05 ges
                     gender 1 54 2.406 1.27e-01 0.023000
stress 2 54 21.166 1.63e-07 * 0.288000
 9 ## 1
10 ## 2
                     stress 2 54 21.166 1.63e-07
11 ## 3
                     time 1 54 0.063 8.03e-01
                                                              0.000564
12 ## 4 gender:stress 2 54 1.554 2.21e-01 0.029000
13 ## 5 gender:time 1 54 4.730 3.40e-02 * 0.041000
14 ## 6 stress:time 2 54 1.821 1.72e-01 0.032000
15 ## 7 gender:stress:time 2 54 6.101 4.00e-03
                                                            * 0.098000
```

There was a statistically significant three-way interaction between time, gender, and stress F(2, 54) = 6.10, p = 0.004.

Post-hoc tests

If there is a significant three-way interaction effect, you can decompose it into:

- Simple two-way interaction: run two-way interaction at each level of third variable,
- Simple simple main effect: run one-way model at each level of second variable, and
- **simple simple pairwise comparisons**: run pairwise or other post-hoc comparisons if necessary.

If you do not have a statistically significant three-way interaction, you need to determine whether you have any statistically significant two-way interaction from the ANOVA output. A significant two-way interaction can be followed up by a simple main effect analysis, which can be followed up by simple pairwise comparisons if significant.

In this section we'll describe the procedure for a significant three-way interaction.

Compute simple two-way interaction

You are free to decide which two variables will form the simple two-way interactions and which variable will act as the third variable.

In the following R code, we have considered the simple two-way interaction of <code>gender*stress</code> at each level of <code>time</code>.

Group the data by time (the within-subject factors) and analyze the simple two-way interaction between gender and stress, which are the between-subjects factors.

```
# two-way interaction at each time levels
two.way <- performance %>%
3
   group_by(time) %>%
4
   anova_test(dv = score, wid = id, between = c(gender, stress))
5 two.way
6 ## # A tibble: 6 x 8
7 ## time Effect
11 ## 3 t1 gender:stress 2 54 2.12 0.131 ""
                                              0.073
12  ## 4 t2  gender
13  ## 5 t2  stress
                      1 54 5.97 0.018 *
                                              0.1
                      2 54 9.60 0.000271 *
                                              0.262
14 ## 6 t2 gender:stress 2 54 4.95 0.011 *
                                                0.155
```

There was a statistically significant simple two-way interaction of gender and stress at t2, F(2, 54) = 4.95, p = 0.011, but not at t1, F(2, 54) = 2.12, p = 0.13.

Note that, statistical significance of a simple two-way interaction was accepted at a Bonferroniadjusted alpha level of 0.025. This corresponds to the current level you declare statistical significance at (i.e., p < 0.05) divided by the number of simple two-way interaction you are computing (i.e., 2).

Compute simple simple main effects

A statistically significant simple two-way interaction can be followed up with **simple simple main effects**.

In our example, you could therefore investigate the effect of stress on the performance score at every level of gender or investigate the effect of gender at every level of stress.

Note that, you will only need to do this for the simple two-way interaction for "t2" as this was the only simple two-way interaction that was statistically significant.

Group the data by time and gender, and analyze the simple main effect of stress on performance score:

```
1 stress.effect <- performance %>%
2
  group_by(time, gender) %>%
3
   anova_test(dv = score, wid = id, between = stress)
  stress.effect %>% filter(time == "t2")
4
5
  ## # A tibble: 2 x 9
       gender time Effect DFn DFd F
                                          p `p<.05` ges
6
7
  ## <fct> <fct> <chr> <dbl> <dbl> <dbl> <dbl> <dbl> <chr> <dbl>
  ## 1 male t2 stress 2 27 1.57 0.227 ""
8
                                                      0.104
9 ## 2 female t2 stress 2 27 10.5 0.000416 *
                                                     0.438
```

In the table above, we only need the results for time = t2. Statistical significance of a simple simple main effect was accepted at a Bonferroni-adjusted alpha level of 0.025, that is 0.05 divided y the number of simple simple main effects you are computing (i.e., 2).

There was a statistically significant simple simple main effect of stress on the performance score for female at t2 time point, F(2, 27) = 10.5, p = 0.0004, but not for males, F(2, 27) = 1.57, p = 0.23.

Compute simple simple comparisons

A statistically significant simple simple main effect can be followed up by **multiple pairwise comparisons** to determine which group means are different.

Note that, you will only need to concentrate on the pairwise comparison results for female, because the effect of stress was significant for female only in the previous section.

Group the data by time and gender, and perform pairwise comparisons between stress levels with Bonferroni adjustment:

```
1  # Fit pairwise comparisons
 pwc <- performance %>%
 3
      group_by(time, gender) %>%
 4
      pairwise_t_test(score ~ stress, p.adjust.method = "bonferroni") %>%
 5
      select(-p, -p.signif) # Remove details
 6 # Focus on the results of "female" at t2
 7 pwc %>% filter(time == "t2", gender == "female")
 8 ## # A tibble: 3 x 9
    ## gender time .y. group1 group2 n1 n2
 9
                                                                                 p.adj p.adj.signif
10 ## <fct> <fct> <chr> <chr> <chr> <int> <int> <int>
                                                                                <dbl> <chr>

      11
      ## 1 female t2
      score low
      moderate
      10
      10 0.323
      ns

      12
      ## 2 female t2
      score low
      high
      10 10 0.000318
      ***

      13
      ## 3 female t2
      score moderate high
      10 0.0235
      *

                                                moderate 10 10 0.323
```

For female, the mean performance score was statistically significantly different between low and high stress levels (p < 0.001) and between moderate and high stress levels (p = 0.023).

There was no significant difference between low and moderate stress groups (p = 0.32)

Report

A three-way mixed ANOVA was performed to evaluate the effects of gender, stress and time on performance score.

There were no extreme outliers, as assessed by box plot method. The data was normally distributed, as assessed by Shapiro-Wilk's test of normality (p > 0.05). There was homogeneity of variances (p > 0.05) as assessed by Levene's test of homogeneity of variances.

There was a statistically significant three-way interaction between gender, stress and time, F(2, 54) = 6.10, p = 0.004.

For the simple two-way interactions and simple simple main effects, a Bonferroni adjustment was applied leading to statistical significance being accepted at the p < 0.025 level.

There was a statistically significant simple two-way interaction between gender and stress at time point t2, F(2, 54) = 4.95, p = 0.011, but not at t1, F(2, 54) = 2.12, p = 0.13.

There was a statistically significant simple simple main effect of stress on the performance score for female at t2 time point, F(2, 27) = 10.5, p = 0.0004, but not for males, F(2, 27) = 1.57, p = 0.23.

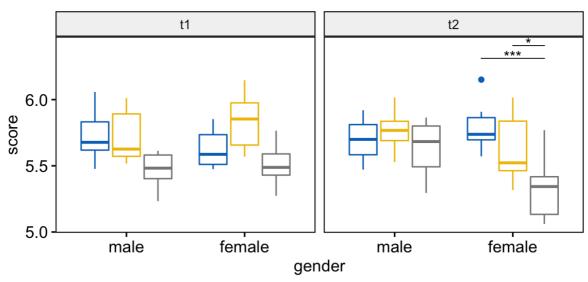
All simple simple pairwise comparisons were run between the different stress groups for female at time point t2. A Bonferroni adjustment was applied.

The mean performance score was statistically significantly different between low and high stress levels (p < 0.001) and between moderate and high stress levels (p = 0.024). There was no significant difference between low and moderate stress groups (p = 0.32).

```
# Visualization: box plots with p-values
1
2
   pwc <- pwc %>% add_xy_position(x = "gender")
3
   pwc.filtered <- pwc %>% filter(time == "t2", gender == "female")
4
   bxp +
5
     stat_pvalue_manual(pwc.filtered, tip.length = 0, hide.ns = TRUE) +
6
7
       subtitle = get_test_label(res.aov, detailed = TRUE),
8
       caption = get_pwc_label(pwc)
9
```

Anova,
$$F(2,54) = 6.1$$
, $p = 0.004$, $\eta_g^2 = 0.1$





pwc: T test; p.adjust: Bonferroni

Three-way Mixed ANOVA: 1 between- and 2 withinsubjects factors

This section describes how to compute the three-way mixed ANOVA, in R, for a situation where you have **one between-subjects factor and two within-subjects factors**. For example, you might want to understand how weight loss score differs in individuals doing exercises vs not doing exercises over three time points (t1, t2, t3) depending on participant diets (diet:no and diet:yes).

Data preparation

We'll use the weightloss dataset available in the datarium package. This dataset was originally created for three-way repeated measures ANOVA. However, for our example in this article, we'll modify slightly the data so that it corresponds to a three-way mixed design.

A researcher wanted to assess the effect of time on weight loss score depending on exercises programs and diet.

The weight loss score was measured in two different groups: a group of participants doing exercises (exercises:yes) and in another group not doing exercises (excises:no).

Each participant was also enrolled in two trials: (1) no diet and (2) diet. The order of the trials was counterbalanced and sufficient time was allowed between trials to allow any effects of previous trials to have dissipated.

Each trial lasted 9 weeks and the weight loss score was measured at the beginning of each trial (t1), at the midpoint of each trial (t2) and at the end of each trial (t3).

In this study design, 24 individuals were recruited. Of these 24 participants, 12 belongs to the exercises:no group and 12 were in exercises:yes group. The 24 participants were enrolled in two successive trials (diet:no and diet:yes) and the weight loss score was repeatedly measured at three time points.

In this setting, we have:

- one dependent (or outcome) variable: score
- One between-subjects factor: exercises
- two within-subjects factors: diet end time

Three-way mixed ANOVA can be performed in order to determine whether there is a significant interaction between diet, exercises and time on the weight loss score.

Load the data and inspect some random rows by group:

```
1  # Load the original data
2 # Wide format
data("weightloss", package = "datarium")
4 # Modify it to have three-way mixed design
5 weightloss <- weightloss %>%
   mutate(id = rep(1:24, 2)) # two trials
7 # Show one random row by group
8 set.seed(123)
   weightloss %>% sample_n_by(diet, exercises, size = 1)
9
10 ## # A tibble: 4 x 6
        id diet exercises t1 t2 t3
11 ##
13 ## 1 4 no no
                         11.1 9.5 11.1
14 ## 2 22 no yes
                         10.2 11.8 17.4
15 ## 3
         5 yes no
                         11.6 13.4 13.9
16 ## 4 23 yes yes 12.7 12.7 15.1
17 | # Gather the columns t1, t2 and t3 into long format.
18 # Convert id and time into factor variables
   weightloss <- weightloss %>%
19
   gather(key = "time", value = "score", t1, t2, t3) %>%
20
    convert_as_factor(id, time)
21
22 # Inspect some random rows of the data by groups
23
   set.seed(123)
24 | weightloss %>% sample_n_by(diet, exercises, time, size = 1)
25 | ## # A tibble: 12 x 5
26 | ## id diet exercises time score
28 ## 1 4 no no
                        t1 11.1
                      t2 10.7
t3 12.3
29 ## 2 10 no no
   ## 3 5 no no
30
```

```
31  ## 4 23  no  yes  t1  10.2

32  ## 5 24  no  yes  t2  13.2

33  ## 6 13  no  yes  t3  15.8

34  ## # ... with 6 more rows
```

Summary statistics

Group the data by exercises, diet and time, and then compute some summary statistics of the score variable: mean and sd (standard deviation)

```
weightloss %>%
group_by(exercises, diet, time) %>%
get_summary_stats(score, type = "mean_sd")

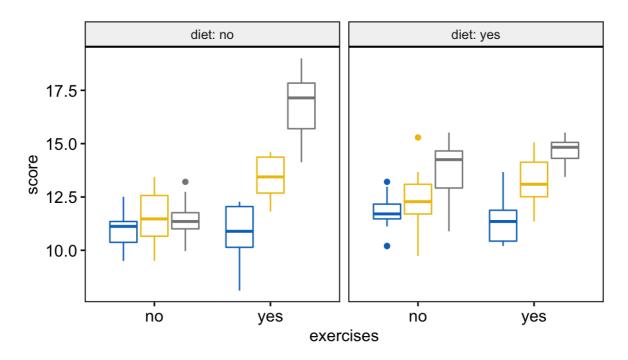
## # A tibble: 12 x 7

## diet exercises time variable n mean sd
## <fct> <fct> <fct> <chr> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <br/> <br/> ## 1 no no t1 score 12 10.9 0.868
## 2 no no t2 score 12 11.6 1.30
## 3 no no t3 score 12 11.4 0.935
## 4 yes no t1 score 12 11.7 0.938
## 5 yes no t2 score 12 12.4 1.42
## 6 yes no t3 score 12 13.8 1.43
## # ... with 6 more rows
```

Visualization

Create box plots of weightloss score by exercises groups, colored by time points and faceted by diet trials:

```
bxp <- ggboxplot(
  weightloss, x = "exercises", y = "score",
  color = "time", palette = "jco",
  facet.by = "diet", short.panel.labs = FALSE
  )
  bxp</pre>
```



Check assumptions

Outliers

```
weightloss %>%
      group_by(diet, exercises, time) %>%
2
      identify_outliers(score)
    ## # A tibble: 5 x 7
                                      score is.outlier is.extreme
         diet exercises time id
         <fct> <fct>
                         <fct> <fct> <dbl> <lgl>
                                                       <1q1>
    ## 1 no
                          t3
                                2
                                       13.2 TRUE
                                                       FALSE
                                       10.2 TRUE
                                                       FALSE
    ## 2 yes
               no
                          t1
    ## 3 yes
                          t1
                                3
                                       13.2 TRUE
                                                       FALSE
10
    ## 4 yes
               no
                          t1
                                4
                                       10.2 TRUE
                                                       FALSE
    ## 5 yes
                          t2
                                10
                                       15.3 TRUE
                                                       FALSE
               no
```

There were no extreme outliers.

Normality assumption

Compute Shapiro-Wilk test for each combinations of factor levels:

```
weightloss %>%
group_by(diet, exercises, time) %>%
shapiro_test(score)

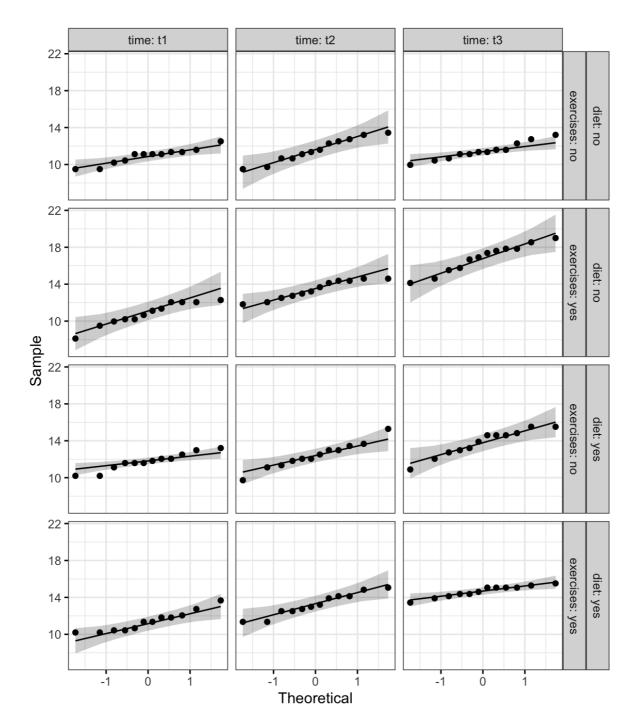
## # A tibble: 12 x 6

## diet exercises time variable statistic p
## * * *fct > * *fct > * *chr > * * *dbl > *dbl > * *dbl > *dbl > * *dbl > *dbl > * *dbl > *dbl > * *dbl > * *dbl > *dbl > * *dbl > *
```

The weight loss score was normally distributed (p > 0.05), as assessed by Shapiro-Wilk's test of normality.

Create QQ plot for each cell of design:

```
ggqqplot(weightloss, "score", ggtheme = theme_bw()) +
facet_grid(diet + exercises ~ time, labeller = "label_both")
```



From the plot above, as all the points fall approximately along this reference line, we can assume normality.

Homogneity of variance assumption

Compute the Levene's test after grouping the data by diet and time categories:

There was homogeneity of variances for all cells (p > 0.05), except for the condition diet:yes at time:t3 (p = 0.034), as assessed by Levene's test of homogeneity of variance.

Note that, if you do not have homogeneity of variances, you can try to transform the outcome (dependent) variable to correct for the unequal variances.

If group sample sizes are (approximately) equal, run the three-way mixed ANOVA anyway because it is somewhat robust to heterogeneity of variance in these circumstances.

It's also possible to perform robust ANOVA test using the WRS2 R package.

Assumption of sphericity

As mentioned in the two-way mixed ANOVA section, the Mauchly's test of sphericity and the sphericity corrections are internally done using the R function anova_test() and get_anova_table() [rstatix package].

Computation

```
1 res.aov <- anova_test(</pre>
 2
    data = weightloss, dv = score, wid = id,
 3
    between = exercises, within = c(diet, time)
 4
    )
 5 get_anova_table(res.aov)
 6 ## ANOVA Table (type II tests)
 7 ##
            Effect DFn DFd F p p<.05 ges
exercises 1 22 38.771 2.88e-06 * 0.284
 8 ##
9 ## 1
10 ## 2
                   diet 1 22 7.912 1.00e-02
                                                      * 0.028
             time 2 44 82.199 1.38e-15
                                                     * 0.541
11 ## 3
12 ## 4 exercises:diet 1 22 51.698 3.31e-07
                                                     * 0.157
            exercises:time 2 44 26.222 3.18e-08 * 0.274
13 ## 5
14 ## 6 diet:time 2 44 0.784 4.63e-01 0.013
15 ## 7 exercises:diet:time 2 44 9.966 2.69e-04 * 0.147
```

From the output above, it can be seen that there is a statistically significant three-way interactions between exercises, diet and time F(2, 44) = 9.96, p = 0.00027.

Note that, if the three-way interaction is not statistically significant, you need to consult the two-way interactions in the output.

In our example, there was a statistically significant two-way exercises diet interaction (p < 0.0001), and two-way exercises time (p < 0.0001). The two-way diet*time interaction was not statistically significant (p = 0.46).

Post-hoc tests

If there is a significant three-way interaction effect, you can decompose it into:

- Simple two-way interaction: run two-way interaction at each level of third variable,
- Simple simple main effect: run one-way model at each level of second variable, and
- **simple simple pairwise comparisons**: run pairwise or other post-hoc comparisons if necessary.

If you do not have a statistically significant three-way interaction, you need to determine whether you have any statistically significant two-way interaction from the ANOVA output. You can follow up a significant two-way interaction by simple main effects analyses and pairwise comparisons between groups if necessary.

In this section we'll describe the procedure for a significant three-way interaction.

Compute simple two-way interaction

In this example, we'll consider the diet*time interaction at each level of exercises. Group the data by exercises and analyze the simple two-way interaction between diet and time:

```
1 # Two-way ANOVA at each exercises group level
  2 two.way <- weightloss %>%
  3
         group_by(exercises) %>%
  4
         anova_test(dv = score, wid = id, within = c(diet, time))
  5 two.way
  6 | ## # A tibble: 2 x 2
  7 ## exercises anova
  8 ## <fct> 1ist>
  9 ## 1 no
                            <anov_tst>
 10 ## 2 yes
                          <anov_tst>
 11 | # Extract anova table
 12 get_anova_table(two.way)
 13 ## # A tibble: 6 x 8
## # A CLOBIE: 6 x 8

## # A CLOBIE: 6 x 8

## exercises Effect DFn DFd F p \( \)p<.05\\ ges

## cfct> cchr> cdbl> cdbl> cdbl> cchr> cdbl>
## 1 no diet 1 11 56.4 1.18e- 5 * 0.262

## 2 no time 2 22 5.90 9.00e- 3 * 0.181

## 3 no diet:time 2 22 2.91 7.60e- 2 "" 0.09

## 4 yes diet 1 11 8.60 1.40e- 2 * 0.066

## 5 yes time 2 22 148. 1.73e-13 * 0.746

## 6 yes diet:time 2 22 7.81 3.00e- 3 * 0.216
```

There was a statistically significant simple two-way interaction between diet and time for exercises:yes group, F(2, 22) = 7.81, p = 0.0027, but not for exercises:no group, F(2, 22) = 2.91, p = 0.075.

Note that, statistical significance of a simple two-way interaction was accepted at a Bonferroniadjusted alpha level of 0.025. This corresponds to the current level you declare statistical significance at (i.e., p < 0.05) divided by the number of simple two-way interaction you are computing (i.e., 2).

Compute simple simple main effect

A statistically significant simple two-way interaction can be followed up with **simple simple main effects**.

In our example, you could therefore investigate the effect of time on the weight loss score at every level of diet and/or investigate the effect of diet at every level of time.

Note that, you will only need to do this for the simple two-way interaction for "exercises:yes" group, as this was the only simple two-way interaction that was statistically significant.

Group the data by exercises and diet, and analyze the simple main effect of time:

```
time.effect <- weightloss %>%
group_by(exercises, diet) %>%
anova_test(dv = score, wid = id, within = time) %>%
get_anova_table()
time.effect %>% filter(exercises == "yes")
## # A tibble: 2 x 9
## diet exercises Effect DFn DFd F p `p<.05` ges
## <fct> <chr> <dbl> <dbl> <dbl> <dbl> <chr> <dbl> = ## 1 no yes time 2 22 78.8 9.30e-11 * 0.801
## 2 yes yes time 2 22 30.9 4.06e- 7 * 0.655
```

In the table above, we only need the results for exercises = yes. Statistical significance of a simple simple main effect was accepted at a Bonferroni-adjusted alpha level of 0.025, that is 0.05 divided y the number of simple simple main effects you are computing (i.e., 2).

The simple simple main effect of time on weight loss score was statistically significant under exercises condition for both diet:no (F(2,22) = 78.81, p < 0.0001) and diet:yes (F(2,22) = 30.92, p < 0.0001) groups.

Compute simple simple comparisons

A statistically significant simple simple main effect can be followed up by **multiple pairwise comparisons** to determine which group means are different.

Recall that, you will only need to concentrate on the pairwise comparison results for exercises:yes.

Group the data by exercises and diet, and perform pairwise comparisons between time points with Bonferroni adjustment. Paired t-test is used:

```
1 | # compute pairwise comparisons
 pwc <- weightloss %>%
 3
    group_by(exercises, diet) %>%
    pairwise_t_test(
4
      score ~ time, paired = TRUE,
p.adjust.method = "bonferroni"
5
6
7
       ) %>%
    select(-statistic, -df) # Remove details
8
9 # Focus on the results of exercises:yes group
10 pwc %>% filter(exercises == "yes") %>%
11
    select(-p) # Remove p column
12
    ## # A tibble: 6 x 9
```

13			exercises	.у.	group1	group2	n1	n2	p.adj	
14		j.signi <fct></fct>	<fct></fct>	<chr></chr>	<chr></chr>	<chr></chr>	<int></int>	<int></int>	<dbl></dbl>	<chr></chr>
15	## 1	no	yes	score	t1	t2	12	12	0.000741	***
16	## 2	no	yes	score	t1	t3	12	12	0.000000121	***
17	## 3	no	yes	score	t2	t3	12	12	0.000257	***
18	## 4	yes	yes	score	t1	t2	12	12	0.01	**
19	## 5	yes	yes	score	t1	t3	12	12	0.00000124	***
20	## 6	yes	yes	score	t2	t3	12	12	0.02	*

All simple simple pairwise comparisons were run between the different time points under exercises condition (i.e., exercises:yes) for diet:no and diet:yes trials. A Bonferroni adjustment was applied.

The mean weight loss score was significantly different in all time point comparisons when exercises are performed (p < 0.05).

Report

A three-way mixed ANOVA was performed to evaluate the effects of diet, exercises and time on weight loss.

There were no extreme outliers, as assessed by box plot method. The data was normally distributed, as assessed by Shapiro-Wilk's test of normality (p > 0.05). There was homogeneity of variances (p > 0.05) as assessed by Levene's test of homogeneity of variances. For the three-way interaction effect, Mauchly's test of sphericity indicated that the assumption of sphericity was met (p > 0.05).

There was a statistically significant three-way interaction between exercises, diet and time F(2, 44) = 9.96, p < 0.001.

For the simple two-way interactions and simple simple main effects, a Bonferroni adjustment was applied leading to statistical significance being accepted at the p < 0.025 level.

There was a statistically significant simple two-way interaction between diet and time for exercises:yes group, F(2, 22) = 7.81, p = 0.0027, but not for exercises:no group, F(2, 22) = 2.91, p = 0.075.

The simple simple main effect of time on weight loss score was statistically significant under exercises condition for both diet:no (F(2,22) = 78.81, p < 0.0001) and diet:yes (F(2,22) = 30.92, p < 0.0001) groups.

All simple simple pairwise comparisons were run between the different time points under exercises condition (i.e., exercises:yes) for diet:no and diet:yes trials. A Bonferroni adjustment was applied. The mean weight loss score was significantly different in all time point comparisons when exercises are performed (p < 0.05).

```
# Visualization: box plots with p-values
pwc <- pwc %>% add_xy_position(x = "exercises")
pwc.filtered <- pwc %>% filter(exercises == "yes")

bxp +

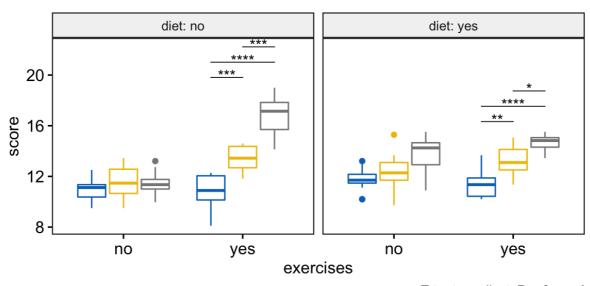
stat_pvalue_manual(pwc.filtered, tip.length = 0, hide.ns = TRUE) +

labs(
subtitle = get_test_label(res.aov, detailed = TRUE),
caption = get_pwc_label(pwc)

)
```

Anova,
$$F(2,44) = 9.97$$
, $p = 0.00027$, $\eta_g^2 = 0.15$

time 🖨 t1 ⊨ t2 🖨 t3



pwc: T test; p.adjust: Bonferroni

Summary

This article describes how to compute and interpret mixed ANOVA in R. We also explain the assumptions made by mixed ANOVA tests and provide practical examples of R codes to check whether the test assumptions are met.