11 Two-way ANOVA

Learning outcomes

Questions

- When is the use of a two-way ANOVA appropriate?
- How do I perform this in R?

Objectives

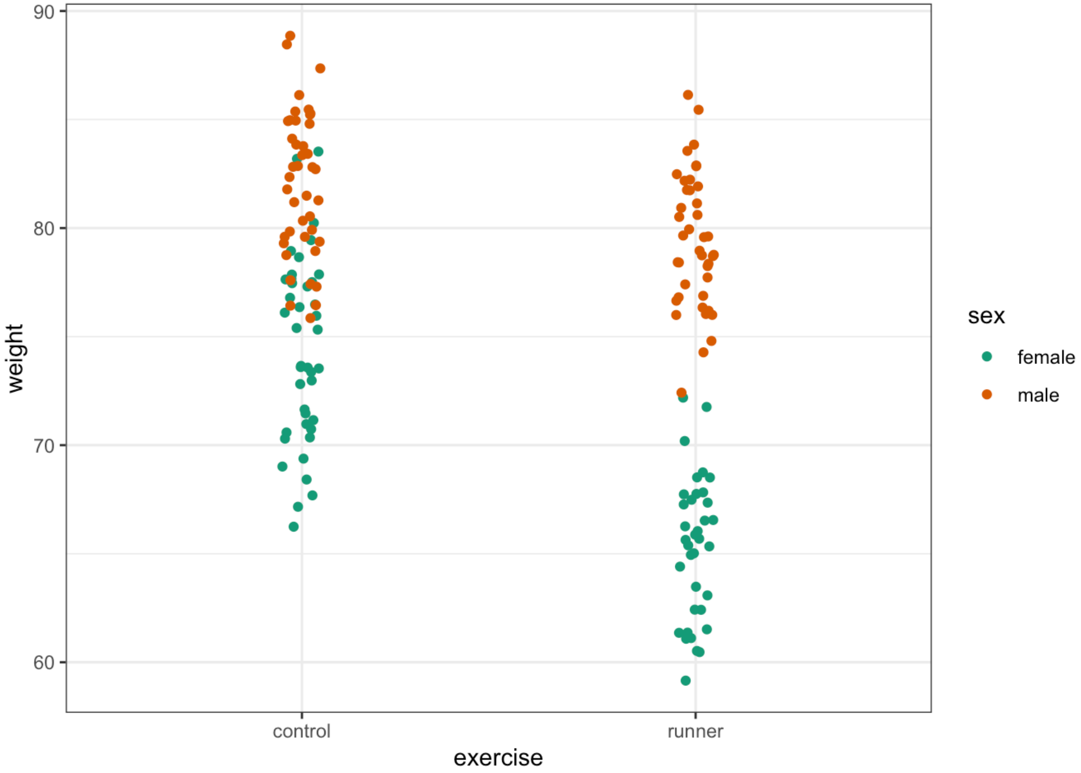
- Be able to perform a two-way ANOVA in R
- Understand the concept of interaction between two predictor variables
- Be able to plot interactions in R

11.1 Libraries and functions

Click to expand

11.2 Purpose and aim

A two-way analysis of variance is used when we have two categorical predictor variables (or factors) and a single continuous response variable. For example, when we are looking at how body `weight` (continuous response variable in kilograms) is affected by sex (categorical variable, `male` or `female`) and `exercise` type (categorical variable, `control` or `runner`).



When analysing these type of data there are two things we want to know:

1. Does either of the predictor variables have an effect on the response variable i.e. does sex affect body weight? Or does being a runner affect body weight?
2. Is there any **interaction** between the two predictor variables? An interaction would mean that the effect that exercise has on your weight depends on whether you are male or female rather than being independent of your sex. For example if being male means that runners weigh more than non-runners, but being female means that runners weight less than non-runners then we would say that there was an interaction.

We will first consider how to visualise the data before then carrying out an appropriate statistical test.

11.3 Data and hypotheses

We will recreate the example analysis used in the lecture. The data are stored as a `.csv` file called `data/CS4-exercise.csv`.

11.4 Summarise and visualise

`exercise` is a data frame with three variables; `weight`, `sex` and `exercise`. `weight` is the continuous response variable, whereas `sex` and `exercise` are the categorical predictor variables.

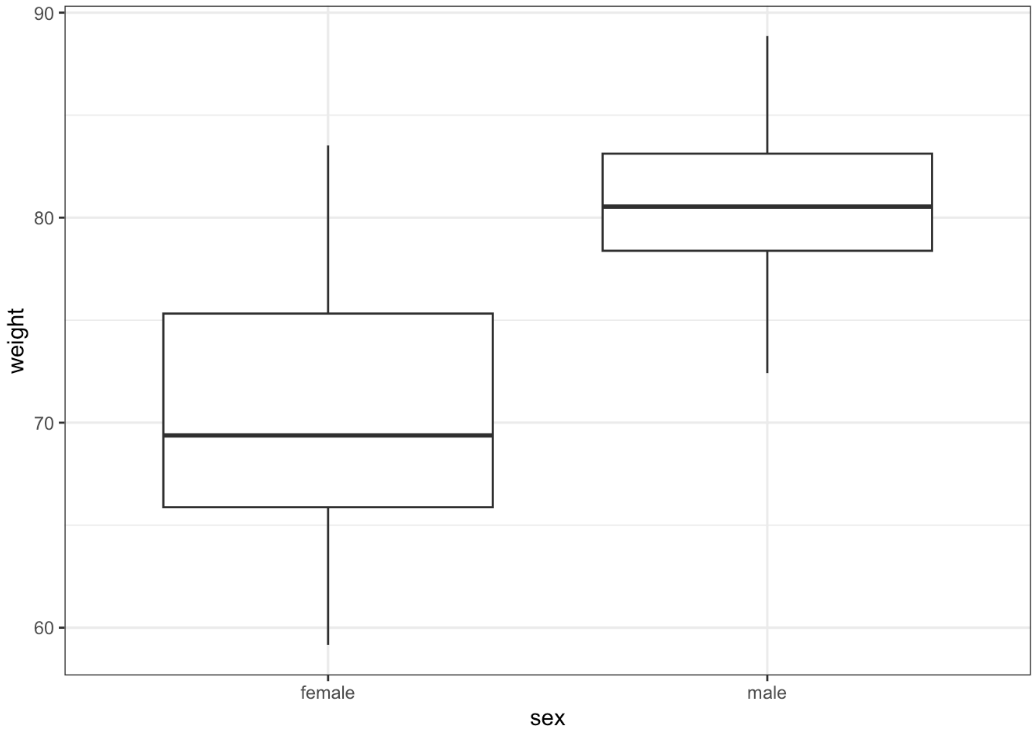
R   Python

First, we read in the data:

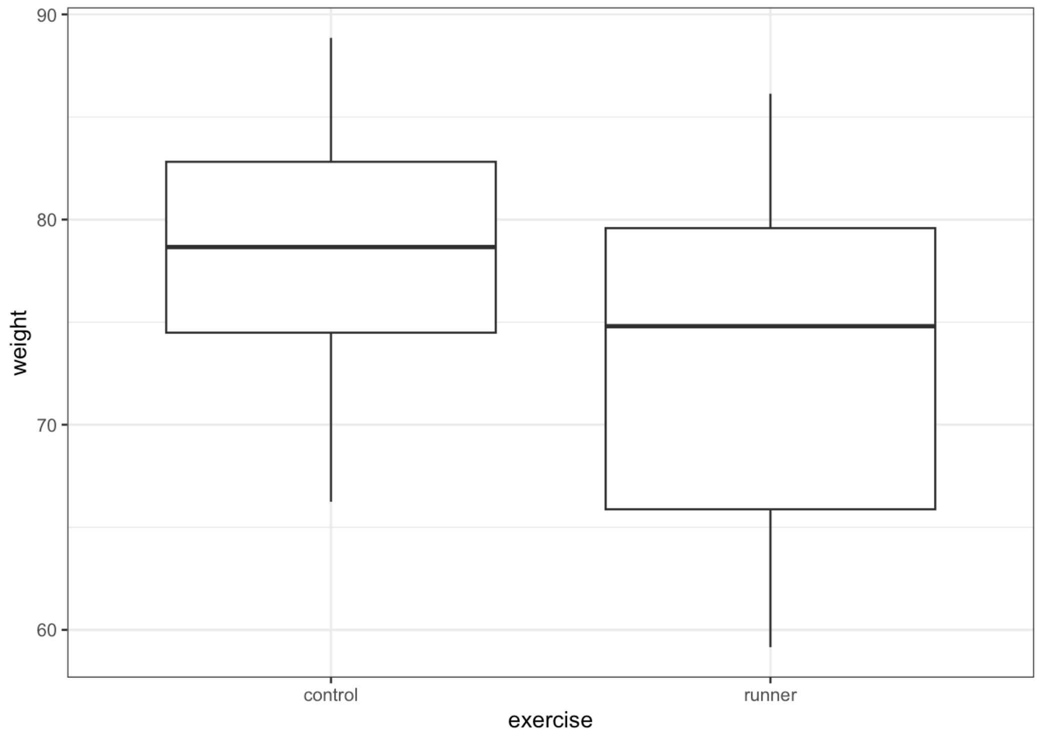
```
exercise <- read_csv("data/CS4-exercise.csv")
```

You can visualise the data with:

```
# visualise the data, sex vs weight
ggplot(exercise,
       aes(x = sex, y = weight)) +
  geom_boxplot()
```



```
# visualise the data, exercise vs weight
ggplot(exercise,
       aes(x = exercise, y = weight)) +
  geom_boxplot()
```

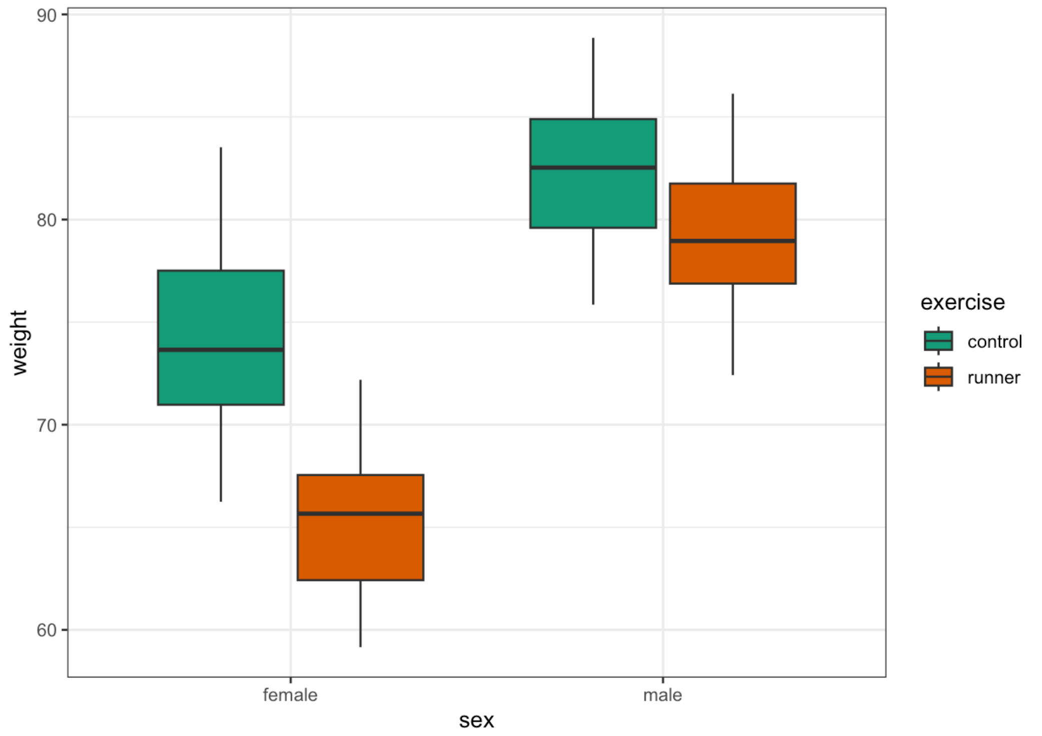


These produce box plots showing the response variable (`weight`) only in terms of one of the predictor variables. The values of the other predictor variable in each case aren't taken into account.

A better way would be to visualise both variables at the same time. We can do this as follows:

R Python

```
ggplot(exercise,
       aes(x = sex, y = weight, fill = exercise)) +
  geom_boxplot() +
  scale_fill_brewer(palette = "Dark2")
```



This produces box plots for all (four) combinations of the predictor variables. We are plotting `sex` on the x-axis; `weight` on the y-axis and filling the box plot by `exercise` regime.

Here I've also changed the default colouring scheme, by using `scale_fill_brewer(palette = "Dark2")`. This uses a colour-blind friendly colour palette (more about the Brewer colour pallete here).

In this example there are only four box plots and so it is relatively easy to compare them and look for any interactions between variables, but if there were more than two groups per categorical variable, it would become harder to spot what was going on.

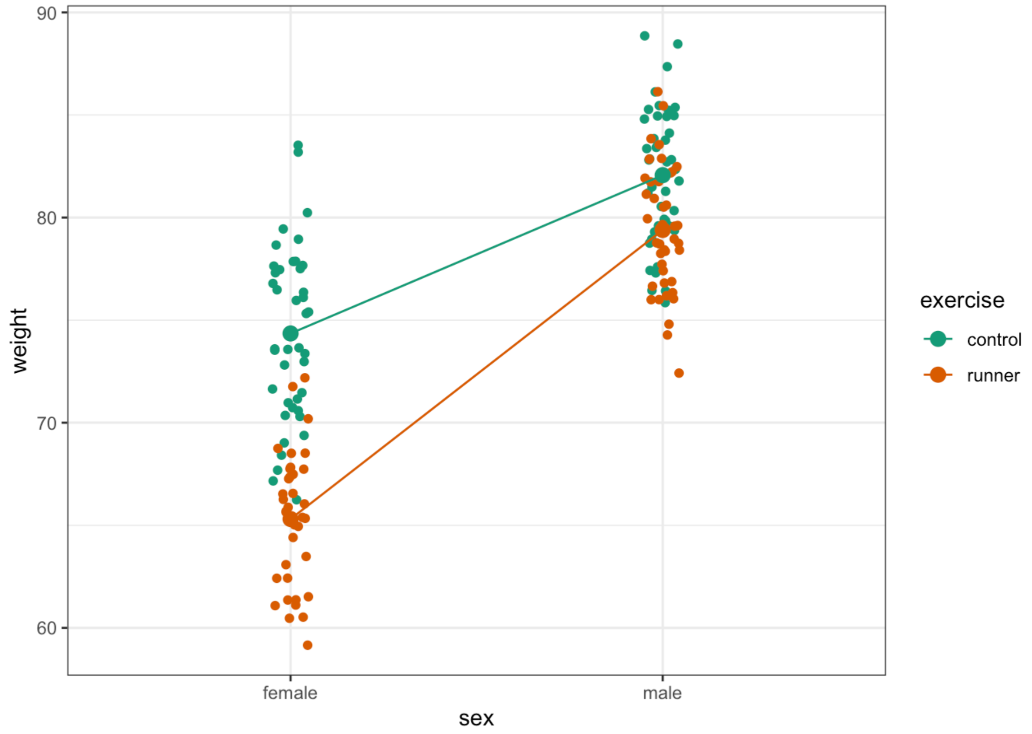
To compare categorical variables more easily we can just plot the group means which aids our ability to look for interactions and the main effects of each predictor variable. This is called an **interaction plot**.

Create an interaction plot:

R Python

We’re adding a bit of jitter to the data, to avoid too much overlap between the data points. We can do this with `geom_jitter()`.

```
ggplot(exercise,
       aes(x = sex, y = weight,
           colour = exercise, group = exercise)) +
  geom_jitter(width = 0.05) +
  stat_summary(fun = mean, geom = "point", size = 3) +
  stat_summary(fun = mean, geom = "line") +
  scale_colour_brewer(palette = "Dark2")
```



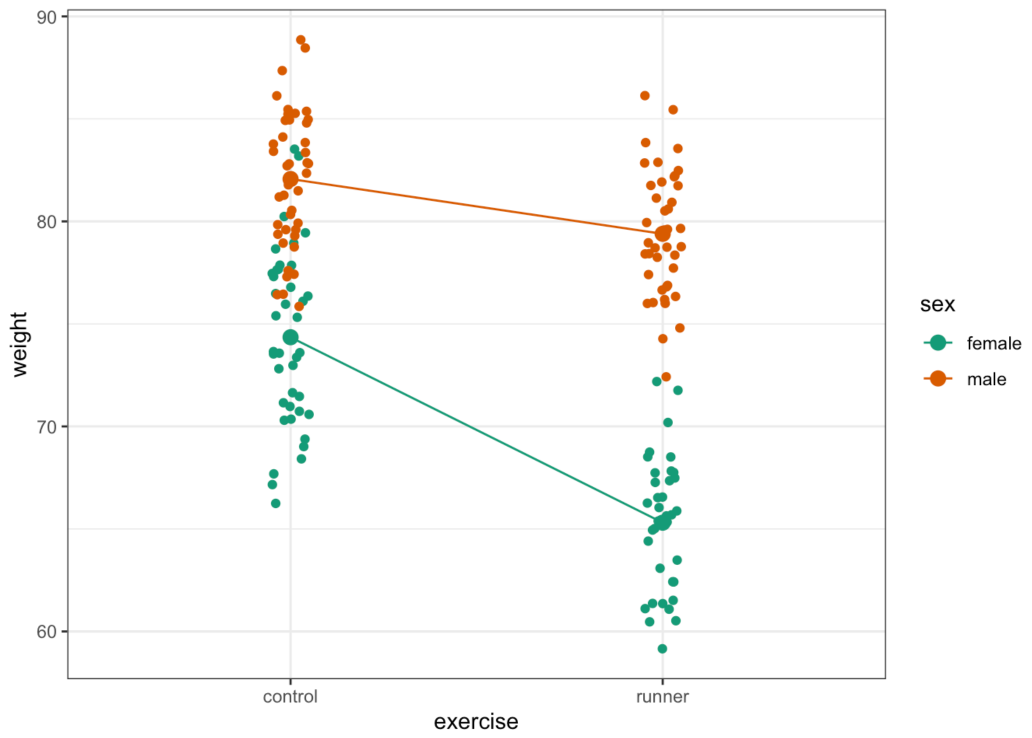
Here we plot `weight` on the y-axis, by `sex` on the x-axis.

- we `colour` the data by `exercise` regime and `group` the data by `exercise` to work out the mean values of each group
- `geom_jitter(width = 0.05)` displays the data, with a tiny bit of random noise, to separate the data points a bit for visualisation
- `stat_summary(fun = mean)` calculates the mean for each group
- `scale_colour_brewer()` lets us define the colour palette

The choice of which categorical factor is plotted on the horizontal axis and which is plotted as different lines is completely arbitrary. Looking at the data both ways shouldn't add anything but often you'll find that you prefer one plot to another.

Plot the interaction plot the other way round:

```r
ggplot(exercise,
       aes(x = exercise, y = weight,
           colour = sex, group = sex)) +
  geom_jitter(width = 0.05) +
  stat_summary(fun = mean, geom = "point", size = 3) +
  stat_summary(fun = mean, geom = "line") +
  scale_colour_brewer(palette = "Dark2")
```



By now you should have a good feeling for the data and could already provide some guesses to the following three questions:

- Does there appear to be any interaction between the two categorical variables?
- If not:

- Does `exercise` have an effect on `weight`?
- Does `sex` have an effect on `weight`?

We can now attempt to answer these three questions more formally using an ANOVA test. We have to test for three things: the interaction, the effect of `exercise` and the effect of `sex`.

11.5 Assumptions

Before we can formally test these things we first need to define the model and check the underlying assumptions. We use the following code to define the model:

R Python

```r
# define the linear model
lm_exercise <- lm(weight ~ sex + exercise + sex:exercise,
                  data = exercise)
```

The `sex:exercise` term is how R represents the concept of an interaction between these two variables.

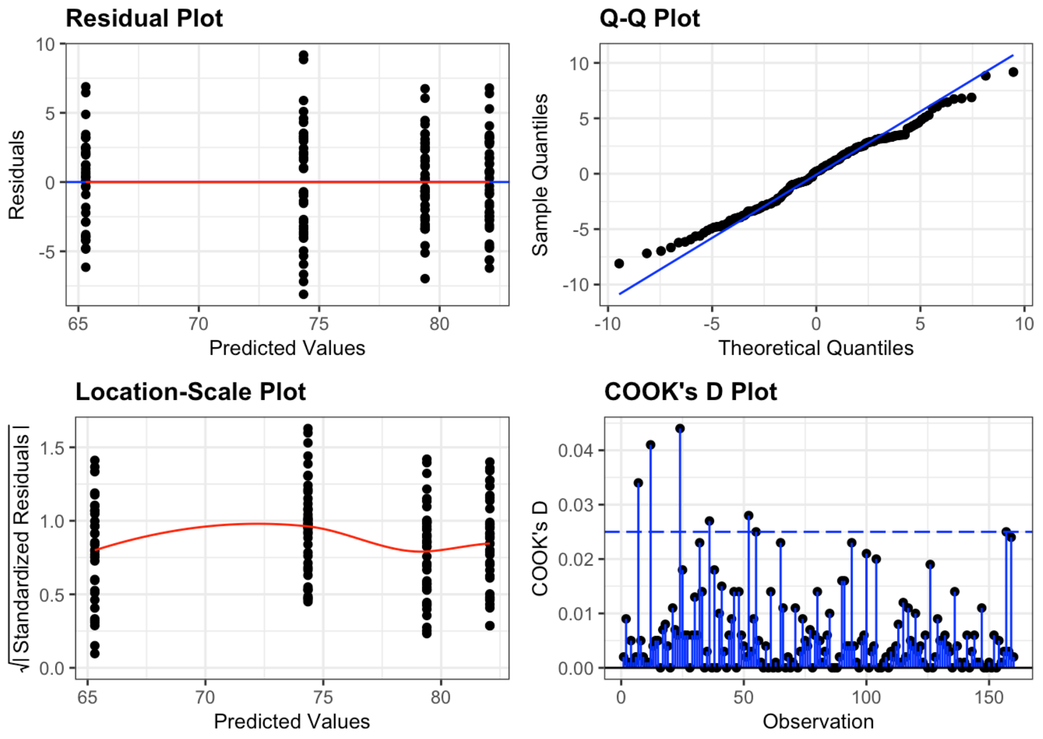
As the two-way ANOVA is a type of linear model we need to satisfy pretty much the same assumptions as we did for a simple linear regression or a one-way ANOVA:

1. The data must not have any systematic pattern to it
2. The residuals must be normally distributed
3. The residuals must have homogeneity of variance
4. The fit should not depend overly much on a single point (no point should have high leverage).

Again, we will check these assumptions visually by producing four key diagnostic plots.

R Python

```
resid_panel(lm_exercise,
            plots = c("resid", "qq", "ls", "cookd"),
            smoother = TRUE)
```



- The Residual plot shows the residuals against the predicted values. There is no systematic pattern here and this plot is pretty good.
- The Q-Q plot allows a visual inspection of normality. Again, this looks OK (not perfect but OK).

- The Location-Scale plot allows us to investigate whether there is homogeneity of variance. This plot is fine (not perfect but fine).
- The Cook's D plot shows that no individual point has a high influence on the model (all values are well below 0.5)

There is a shorthand way of writing:

```
weight ~ sex + exercise + sex:exercise
```

If you use the following syntax:

```
weight ~ sex * exercise
```

Then R interprets it exactly the same way as writing all three terms. You can see this if you compare the output of the following two commands:

```
anova(lm(weight ~ sex + exercise + sex:exercise,
         data = exercise))
```

```
Analysis of Variance Table

Response: weight
              Df Sum Sq Mean Sq F value    Pr(>F)    
sex            1 4510.1  4510.1 366.911 < 2.2e-16 ***
exercise       1 1312.0  1312.0 106.733 < 2.2e-16 ***
sex:exercise   1  404.4   404.4  32.902 4.889e-08 ***
Residuals    156 1917.6    12.3                      
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(lm(weight ~ sex * exercise,
         data = exercise))
```

```
Analysis of Variance Table

Response: weight
```

```
              Df Sum Sq Mean Sq F value    Pr(>F)    
sex            1 4510.1  4510.1 366.911 < 2.2e-16 ***
exercise       1 1312.0  1312.0 106.733 < 2.2e-16 ***
sex:exercise   1  404.4   404.4  32.902 4.889e-08 ***
Residuals    156 1917.6    12.3                      
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

11.6 Implement and interpret test

The assumptions appear to be met well enough, meaning we can implement the ANOVA. We do this as follows (this is probably the easiest bit!):

R | Python

```r
# perform the ANOVA
anova(lm_exercise)
```

```
Analysis of Variance Table

Response: weight
              Df Sum Sq Mean Sq F value    Pr(>F)    
sex            1 4510.1  4510.1 366.911 < 2.2e-16 ***
exercise       1 1312.0  1312.0 106.733 < 2.2e-16 ***
sex:exercise   1  404.4   404.4  32.902 4.889e-08 ***
Residuals    156 1917.6    12.3                      
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

We have a row in the table for each of the different effects that we've asked R to consider. The last column is the important one as this contains the p-values. We need to look at the interaction row first.

`sex:exercise` has a p-value of about 4.89e-08 (which is smaller than 0.05) and so we can conclude that the interaction between `sex` and `exercise` is significant.

This is where we must stop.

The top two lines (corresponding to the effects of `sex` and `exercise`) are meaningless now. This is because the interaction means that we cannot interpret the main effects independently.

In this case, `weight` depends on *and* the sex *and* the exercise regime. This means the effect of `sex` on `weight` is dependent on `exercise` (and vice-versa).

We would report this as follows:

> A two-way ANOVA test showed that there was a significant interaction between the effects of sex and exercise on weight (p = 4.89e-08). Exercise was associated with a small loss of weight in males but a larger loss of weight in females.

11.7 Exercises

11.7.1 Auxin response

Exercise 1

LEVEL: ★★☆

Plant height responses to auxin in different genotypes

These `data/CS4-auxin.csv` data are from a simulated experiment that looks at the effect of the plant hormone auxin on plant height.

The experiment consists of two genotypes: a wild type control and a mutant (`genotype`). The plants are treated with auxin at different concentrations: `none`, `low` and `high`, which are stored in the `concentration` column.

The response variable plant height (`plant_height`) is then measured at the end of their life cycle, in centimeters.

Questions to answer:

- Visualise the data using boxplots and interaction plots.
- Does there appear to be any interaction between `genotype` and `concentration`?
- Carry out a two-way ANOVA test.
- Check the assumptions.
- What can you conclude? (Write a sentence to summarise).

Answer

11.8.1 Tulips

Exercise 2

LEVEL: ★★☆

Blooms and growing conditions

We're sticking with the plant theme and using the `data/CS4-tulip.csv` data set, which contains information on an experiment to determine the best conditions for growing tulips (well someone has to care about these sorts of things!). The average number of flower heads (blooms) were recorded for 27 different plots. Each plot experienced one of three different watering regimes and one of three different shade regimes.

- Investigate how the number of blooms is affected by different growing conditions.

Note: have a look at the data and make sure that they are in the correct format!

Answer

11.9 Summary

Key points

- A two-way ANOVA is used when there are two categorical variables and a single continuous variable
- We can visually check for interactions between the categorical variables by using interaction plots
- The two-way ANOVA is a type of linear model and assumes the following:
    1. the data have no systematic pattern
    2. the residuals are normally distributed
    3. the residuals have homogeneity of variance
    4. the fit does not depend on a single point (no single point has high leverage)