

1 Univariate tests

1.1 One sample t test

Assumption: the data X_1, \dots, X_n follows normal distribution $N(\mu, \sigma^2)$ and are independent and identically distributed (i.i.d.). We would like to know the mean of the normal distribution μ is equal to a particular value μ_0 .

Before we state the decision rule, we define some notations.

Definition 1. Suppose n is the degree of freedom of a student's t distribution. T is a random variable which follows student's t distribution with degree of freedom n . $t_{\alpha,n}$ is the α th quantile of a student's t distribution with degree of freedom n , i.e. $\mathbb{P}(T < t_{\alpha,n}) = \alpha$, where $0 \leq \alpha \leq 1$. \square

Define:

$$\bar{X} = \frac{1}{n} \sum_{i=1}^n X_i, \quad S_X = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (X_i - \bar{X})^2}.$$

Test statistic:

$$T = \frac{\sqrt{n}(\bar{X} - \mu)}{S_X}.$$

α is known as the significance level. It is the probability of rejecting a true H_0 . Usually α is set to be 0.01, 0.05 or 0.1. We formulate the following 3 hypothesis testings:

1. $H_0 : \mu = \mu_0$ vs $H_1 : \mu \neq \mu_0$. Reject H_0 if $|T| > t_{1-\frac{\alpha}{2}, n-1}$.
2. $H_0 : \mu = \mu_0$ vs $H_1 : \mu > \mu_0$. Reject H_0 if $T > t_{1-\alpha, n-1}$.
3. $H_0 : \mu = \mu_0$ vs $H_1 : \mu < \mu_0$. Reject H_0 if $T < t_{\alpha, n-1}$.

In the above three testings, when p -value is smaller than α , we reject H_0 .

Example 1. We take the difference between GABAA before and after exercise for those 4 participants between 24/09/2019 and 28/09/2019. The differences are:

$$X_1 = 0.19, X_2 = 0.09, X_3 = 0.23, X_4 = 0.09.$$

The following R code inputs the data.

```
y<-c(0.19,0.09,0.23,0.09)
```

First, we have to check if the normality assumption is met using the following code.

```
#To test for normality
shapiro.test(y)
```

The following is the output:

```
Shapiro-Wilk normality test
data:  y
W = 0.83785, p-value = 0.1892
```

The p -value is 0.1892, which is greater than any significance level. The normality assumption is met.

We would like to test if the difference is greater than zero using one sample t test. That is,

$$H_0 : \mu = 0 \quad \text{vs} \quad H_1 : \mu > 0.$$

The following code will conduct the one sample t test.

```
#To test for H1: mu > 0 t.test(y,mu=0,alternative="greater")
t.test(y,mu=0,alternative="greater")
```

The following is the output

```
data:  y
t = 4.2146, df = 3, p-value = 0.0122
alternative hypothesis: true mean is greater than 0
95 percent confidence interval:
0.06624318      Inf
sample estimates: mean of x
0.15
```

The p -value is 0.0122. If we set the significance level α to be 0.05, then we reject the null hypothesis and conclude that the GABAA after exercise is greater than GABAA before exercise at 5% significance level.

We can also test for alternative hypotheses $H_1 : \mu \neq 0$ or $H_1 : \mu < 0$ with the following codes.

```
#To test for H1: mu is not equal to 0
t.test(y,mu=0)
```

```
#To test for H1: mu < 0
t.test(y,mu=0,alternative="less")
```

□

1.2 Two sample unpaired t test

Suppose we have two normal samples:

$$X_1, \dots, X_m \sim N(\mu_1, \sigma_1^2)$$

$$Y_1, \dots, Y_n \sim N(\mu_2, \sigma_2^2)$$

X_i 's and Y_j 's are independent. m and n are not necessarily equal.

Assume that $\sigma_1 = \sigma_2$. We can test:

$$H_0 : \mu_1 = \mu_2 \quad \text{vs} \quad H_1 : \mu_1 \neq \mu_2.$$

Example 2. Suppose we randomly select some men and women and measure their weights. Obviously, the men's weights and women's weights are independent. Then, we check for normality.

```
# Shapiro-Wilk normality test for Men's weights
with(my_data, shapiro.test(weight[group == "Man"]))
# Shapiro-Wilk normality test for Women's weights
with(my_data, shapiro.test(weight[group == "Woman"]))
```

The following is the output

```
data:  weight[group == "Man"]
W = 0.86425, p-value = 0.1066
```

```
data:  weight[group == "Woman"]
W = 0.94266, p-value = 0.6101
```

The p -values are greater than 0.1. We may conclude that the data are normally distributed at 10% significance level.

Then, we have to check if the variance of men's weights is equal to that of women's weights

```
#Test for equality of variances
res.ftest <- var.test(weight ~ group, data = my_data)
res.ftest
```

The following is the output:

```
F test to compare two variances
```

```
data: weight by group
```

```
F = 0.36134, num df = 8, denom df = 8, p-value = 0.1714
```

```
alternative hypothesis: true ratio of variances is not equal to 1
```

```
95 percent confidence interval: 0.08150656 1.60191315
```

```
sample estimates: ratio of variances 0.3613398
```

p -value is 0.1714, greater than 0.1. We may conclude that both variances of men's weights and women's weights are equal at 10% significance level.

The following code conducts two sample unpaired t test.

```
# Compute t-test
```

```
res <- t.test(women_weight, men_weight, var.equal = TRUE)
```

```
res
```

The output is:

```
Two Sample t-test
```

```
data: women_weight and men_weight t = -2.7842, df = 16, p-value = 0.01327
```

```
alternative hypothesis: true difference in means is not equal to 0
```

```
95 percent confidence interval: -29.748019 -4.029759
```

```
sample estimates: mean of x mean of y
```

```
52.10000 68.98889
```

p -value is 0.01327, less than 0.05. We may conclude that men's weights are different from women's weights at 5% significance level. \square

1.3 One-way ANOVA

ANOVA is for investigating whether the response variables Y 's of r groups are different significantly. For instance, we would like to investigate whether the weights of male and female are different significantly. Y would be the weight and $r = 2$ since there are two groups, male and female. Here gender is a factor. And there are two factor levels, corresponding to those two genders.

The main objectives of ANOVA are:

1. Determine whether the factor level means are the same.
2. If the factor level means are not the same, examine how they differ.

Here we introduce some statistical settings.

- Number of factor levels: r
- Number of observations for the i th factor level: n_i
- Total number of observations: $n_T = \sum_{i=1}^r n_i$
- Y_{ij} is the j th observation on the response variable for the i th factor level
- ANOVA model is defined as

$$Y_{ij} = \mu_i + \epsilon_{ij}, \quad (1)$$

where:

- Y_{ij} is the value of the response variable in the j th trial for the i th factor level
- μ_i are the parameters
- ϵ_{ij} are independent $N(0, \sigma^2)$, $i = 1, \dots, r; j = 1, \dots, n_i$.

So, we assume that each Y_{ij} follows $N(\mu_i, \sigma^2)$ and are independent from each other.

Example 3. Suppose a company wants to compare the annual sales figures of four teams, A, B, C and D.

There are 2 teammates in team A, 3 teammates in team B, etc. The following data are obtained.

Team	Teammate 1	Teammate 2	Teammate 3
A	12	18	
B	14	12	13
C	19	17	21
D	24	30	

We are interested in testing if the mean of all four teams are equal:

$$H_0 : \mu_A = \mu_B = \mu_C = \mu_D \quad \text{vs} \quad H_1 : H_0 \text{ is not true.}$$

The following code inputs data into R and conduct ANOVA test:

```
#input data
group<-c("A","A","B","B","B","C","C","C","D","D")
sales<-c(12,18,14,12,13,19,17,21,24,30)
data_ex3<-data.frame(group=group,sales=sales)
#to produce boxplot
library("ggpubr")
ggboxplot(data_ex3, x = "group", y = "sales", order = c("A", "B", "C", "D"), ylab = "Sales",
```

```
#ANOVA test
res.aov <- aov(sales ~ group, data = data_ex3)
#Summary of the analysis
summary(res.aov)
```

The output is as follow:

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
group	3	258	86.00	11.22	0.00713 **
Residuals	6	46	7.67		

$p\text{-value} = 0.00713 < 0.01$. We reject the null hypothesis and may conclude that the four teams do not have the same sales at 1% significance level.

Now, the factor level means are not the same, we have to examine how they differ. The following R code will compute the confidence interval of the differences in means.

```
#All pairwise comparisons of factor level means
TukeyHSD(res.aov)
```

The output is:

```
Tukey multiple comparisons of means
95% family-wise confidence level
Fit: aov(formula = sales ~ group, data = data_ex3)
```

\$group	diff	lwr	upr	p adj
B-A	-2	-10.7499087	6.749909	0.8561049
C-A	4	-4.7499087	12.749909	0.4522099
D-A	12	2.4149552	21.585045	0.0190946
C-B	6	-1.8261563	13.826156	0.1304578
D-B	14	5.2500913	22.749909	0.0058766
D-C	8	-0.7499087	16.749909	0.0707158

We tabulate the result in the following table

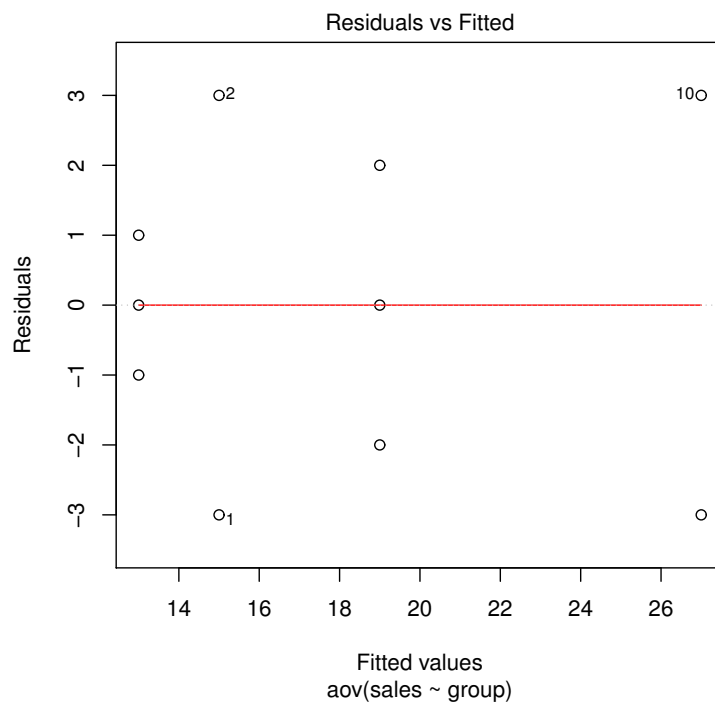
Difference_group	Difference	Lower bound of CI	Upper bound of CI
$\mu_B - \mu_A$	-2	-10.7499087	6.749909
$\mu_C - \mu_A$	4	-4.7499087	12.749909
$\mu_D - \mu_A$	12	2.4149552	21.585045
$\mu_C - \mu_B$	6	-1.8261563	13.826156
$\mu_D - \mu_B$	14	5.2500913	22.749909
$\mu_D - \mu_C$	8	-0.7499087	16.749909

We see that the confidence interval of $\mu_D - \mu_A$ does not contain zero, so $\mu_D - \mu_A$ differs from zero significantly.

We should also check for the equality (homogeneity) of variance of the residuals ($Y_{ij} - \bar{Y}_{i.}$, the difference between the observed value and estimated value) by the following code:

```
#Check homogeneity of variances
plot(res.aov, 1)
```

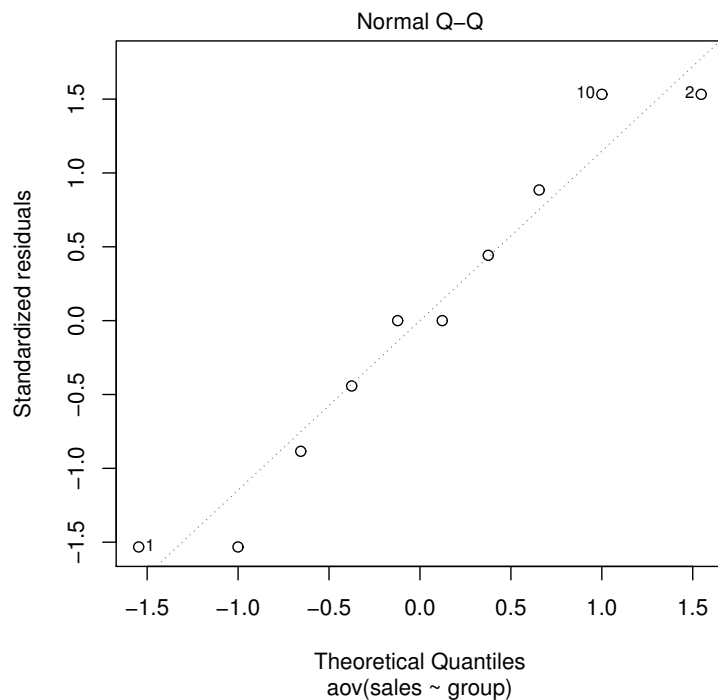
The plot below shows the residuals against the fitted factor level means. It seems that the variance of the residuals is constant for all fitted values.



We should check the normality of the residuals by inspecting a quantile-quantile plot. The following code will generate such plot:

```
#Check normality
```

```
plot(res.aov,2)
```



From the plot, the residuals lie quite closely to the 45 degree line. So, it seems that the normality assumption is met.

□

ff

Example 4. Consider the GABAA data set. In the code, I create two new columns, one of which contains the GABAA measured after exercise (GABAA_post_exercise), another of which contains the difference between GABAA_pre_exercise and GABAA_post_exercise. Conduct one-way ANOVA using gender as the only factor. Treat all observations as if they are independent. Of course it violates the independent assumption since some observations come from the same patient.

The p -value of the variable “sexe” is 0.015, smaller than 0.05. We may conclude that sexe has effect on GABAA_diff.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sexe	1	0.02001	0.020008	8.593	0.015 *
Residuals	10	0.02328	0.002328		

1.4 Two way ANOVA

Two way ANOVA has two factors, factor A and factor B . Factor A can be gender, factor B can be age group (young, middle, old).

- Factor A has a factor levels.
 - i = factor level of factor A ($i = 1, \dots, a$).
 - If we take factor A as gender, then factor A has 2 levels, $a = 2$.
- Factor B has b factor levels.
 - j = factor level of factor B ($j = 1, \dots, b$).
 - If we take factor B as age group, then factor B has 3 levels, $b = 3$.
- Treatment
 - Each combination of factor level i of factor A and factor level j of factor B is a treatment.
 - ab combinations.
 - ab treatments.
- Sample size
 - n = no. of cases of each of the treatment
 - equal sample size
 - Total number of cases, $n_T = abn$.
- Response variable
 - Y_{ijk} = k th observation where A is at i th level and B is at j th level
- The model is

$$Y_{ijk} = \mu_{ij} + \epsilon_{ijk},$$

where

- μ_{ij} is the treatment mean at i th level of factor A and j th level of factor B
- ϵ_{ijk} are independent $N(0, \sigma^2)$
- $i = 1, \dots, a; j = 1, \dots, b; k = 1, \dots, n$.

Example 5. A study of the effects of gender and age on learning of a task are of interest. There are 3 age groups (young, middle, old) and 2 genders (male, female). $a = 2$, $b = 3$, so $ab = 6$ treatments. The mean learning time (in minute) of the treatments are given as follows

Factor A	Factor B (Age)		
(Gender)	Young ($j = 1$)	Middle ($j = 2$)	Old ($j = 3$)
Male ($i = 1$)	$\mu_{11} = 9$	$\mu_{12} = 11$	$\mu_{13} = 16$
Female ($i = 2$)	$\mu_{21} = 9$	$\mu_{22} = 11$	$\mu_{23} = 16$

- $\mu_{11} = 9$, mean learning time for young males is 9 minutes
- $\mu_{22} = 11$, mean learning time for middle-aged females is 11 minutes.
- Mean learning time for men and women are the same for each age group
 - Gender has no effect on mean learning time.
- Mean learning time increases with age for each gender
 - Age has effect on mean learning time.

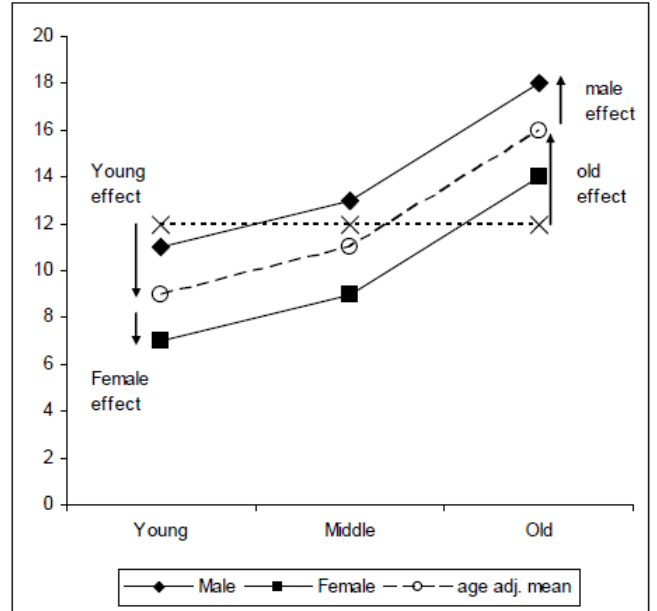
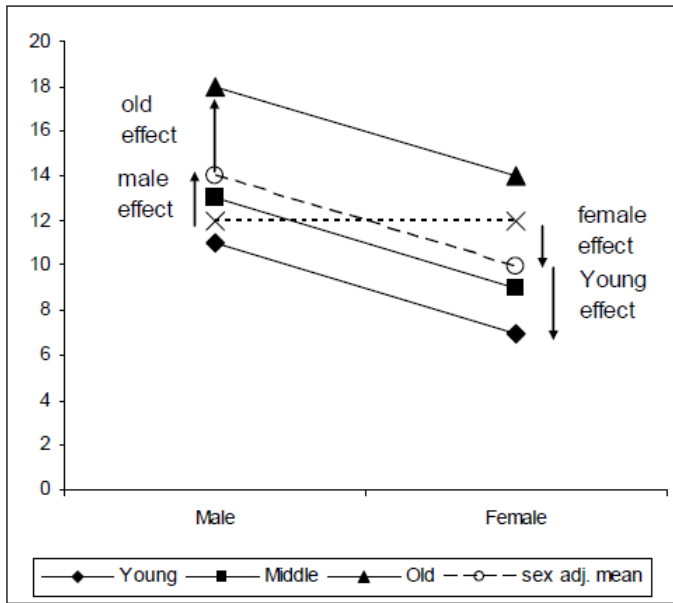
□

Example 6. In this example, gender has effect on learning time. However, age and gender do not interact.

Factor A	Factor B (Age)		
(Gender)	Young ($j = 1$)	Middle ($j = 2$)	Old ($j = 3$)
Male ($i = 1$)	$\mu_{11} = 11$	$\mu_{12} = 13$	$\mu_{13} = 18$
Female ($i = 2$)	$\mu_{21} = 7$	$\mu_{22} = 9$	$\mu_{23} = 14$

- For young, the difference between male and female:
 - $\mu_{11} - \mu_{21} = 4 \neq 0$.
- For middle, the difference between male and female:
 - $\mu_{12} - \mu_{22} = 4 \neq 0$.
- For old, the difference between male and female:
 - $\mu_{13} - \mu_{23} = 4 \neq 0$.
- Main gender effect exists
- For male: $\mu_{11} - \mu_{12} = -2$, $\mu_{12} - \mu_{13} = -5, \dots$

- For female: $\mu_{21} - \mu_{22} = -2, \dots$
- Main age effect exists.
- Do gender and age interact? Does any interaction effect exist?
 - For all factor levels $i = 1, 2$ (gender), the differences between age group are the same
 - * for $i = 1$ (male), young – middle = $\mu_{11} - \mu_{12} = -2$, middle – old = $\mu_{12} - \mu_{13} = -5$.
 - * for $i = 2$ (female), young – middle = $\mu_{21} - \mu_{22} = -2$, middle – old = $\mu_{22} - \mu_{23} = -5$.
 - for all factor levels $j = 1, 2, 3$ (age group), the differences between those two genders are the same.
 - * for $j = 1$ (young), male – female = $\mu_{11} - \mu_{21} = 4$
 - * for $j = 2$ (middle), male – female = $\mu_{12} - \mu_{22} = 4$
 - * for $j = 3$ (old), male – female = $\mu_{13} - \mu_{23} = 4$
- The line plots are parallel, indicating there is no interaction effect between age and gender

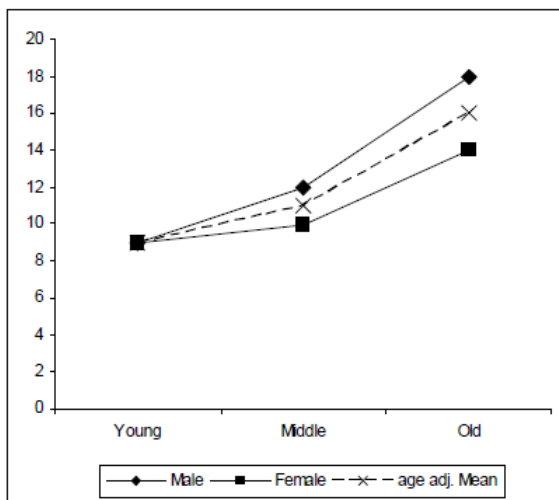


□

Example 7. Now there is interaction effect between age and gender on learning time.

Factor A	Factor B (Age)		
(Gender)	Young ($j = 1$)	Middle ($j = 2$)	Old ($j = 3$)
Male ($i = 1$)	$\mu_{11} = 9$	$\mu_{12} = 12$	$\mu_{13} = 18$
Female ($i = 2$)	$\mu_{21} = 9$	$\mu_{22} = 10$	$\mu_{23} = 14$

- For young age, both male and female have the same mean learning time $\mu_{11} - \mu_{21} = 0$.
- However, as age becomes larger, the difference between learning time for male and female becomes larger.
 - For middle-aged, difference between learning time for male and female is $\mu_{12} - \mu_{22} = 2$.
 - For old-aged, the difference is $\mu_{13} - \mu_{23} = 4$.
- Interaction between age and gender exists.
- The curves are not parallel.



□

Example 8. Here, we'll use the built-in R data set named `ToothGrowth`. It contains data from a study evaluating the effect of vitamin C on tooth growth in Guinea pigs. The experiment has been performed on 60 pigs, where each animal received one of three dose levels of vitamin C (0.5, 1, and 2 mg/day) by one of two delivery methods (supp), orange juice (OJ) or ascorbic acid (a form of vitamin C and coded as VC). Tooth length was measured.

```
# Two-way ANOVA with interaction effect
# These two calls are equivalent
res.aov3 <- aov(len ~ supp * dose, data = my_data)
res.aov3 <- aov(len ~ supp + dose + supp:dose, data = my_data) summary(res.aov3)
```

The output is:

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
supp	1	205.4	205.4	15.572	0.000231	***
dose	2	2426.4	1213.2	92.000	< 2e-16	***
supp:dose	2	108.3	54.2	4.107	0.021860	*
Residuals	54	712.1	13.2			

The p -value of the interaction between delivery method (supp) and dose (supp:dose) is 0.021860, smaller than 0.05. We may reject the null hypothesis and conclude that delivery method and dose interact with each other.

Recall in one way ANOVA, there is only one factor and we consider the difference between **factor levels**. If gender is a factor, then male and female are factor levels.

In two way ANOVA, if the interaction effect is significant, we have to consider the difference between **treatments**. A treatment is a combination of a factor level in factor A and a factor level of factor B . In this example, a dose of 0.5 mg/day and a delivery method of OJ would be a treatment. A dose of 2 mg/day and a delivery method of VJ would be another treatment.

The means of tooth length of all treatments are as follow:

	dose = 0.5	dose = 1.0	dose = 2.0
supp = OJ	13.23	22.7	26.06
supp = VC	7.98	16.77	26.14

For each level of dose, we can compute the confidence intervals for the difference between OJ and VC.

For dose = 0.5, the difference between OJ and VC is $13.23 - 7.98 = 5.25$, and the confidence interval is (1.585949, 8.914051), excludes 0, +ve.

For dose = 1.0, the difference between OJ and VC is $22.7 - 16.77 = 5.93$, and the confidence interval is (2.265949, 9.594051), excludes 0, +ve.

For dose = 2.0, the difference between OJ and VC is $26.06 - 26.14 = -0.08$, and the confidence interval is (-3.744051, 3.584051), includes 0.

□

2 Multivariate tests

What is the difference between univariate and multivariate?

- Univariate, only one dependent variable (IQ)
- Gender is a factor, or explanatory variable:

Student_id	gender	IQ
1	M	100
2	M	101
3	F	110
4	F	90
5	M	93

Multivariate:

- Three dependent variables (math, phy, eng scores) <-multivariate
 - The dependent variables may affect each other. They may not be independent.
- Gender is a factor or explanatory variable

Student_id	gender	Mathematics score	Physics score	English score
1	M	100	100	50
2	F	90	80	50
3	M	80	75	40
4	M	100	100	100
5	F	90	90	100

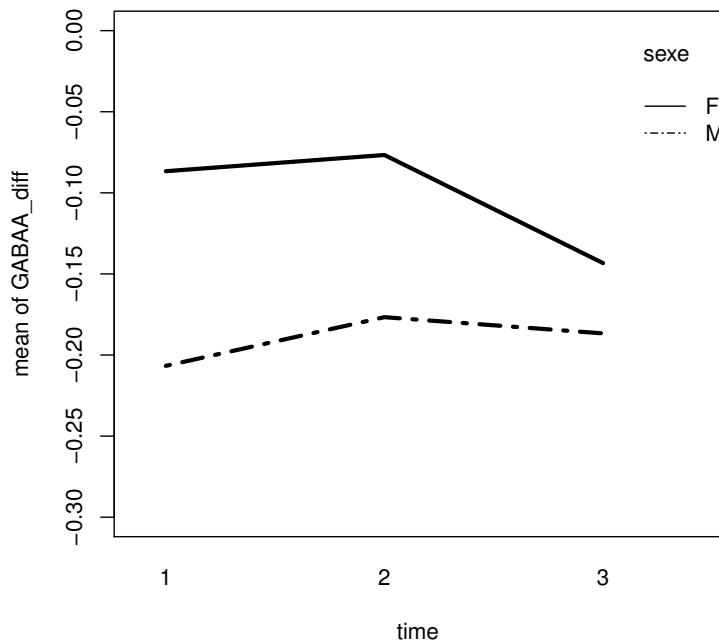
Another example:

We measure the difference between GABAA_pre_exercise and GABAA_post_exercise at three time points for each participant.

- GABAA_diff at time 1, at time 2, at time 3 are three response variables. For each participant, these three variables may not be independent.
- Gender and age are explanatory variables.

Participant_id	gender	age	GABAA_diff at time 1	GABAA_diff at time 2	GABAA_diff at time 3
1	M	young	−0.19	−0.13	−0.21
2	F	young	−0.09	−0.07	−0.12
3	M	young	−0.23	−0.24	−0.17
4	F	young	−0.09	−0.09	−0.22
5	M	old	−0.20	−0.16	−0.18
6	F	old	−0.08	−0.07	−0.09

Example 9. The data set of GABAA is used for one-way MANOVA. I added two more participants (participant_id = 5 and participant_id = 6). Let see how the “profile” looks like.



Profile analysis:

- Are the 2 gender profiles parallel?
 - If yes, do the profiles coincide?
 - * Regardless of whether the profiles coincide or not, are the profiles horizontal?
 - If no, test MANOVA: $H_0 : \boldsymbol{\mu}_M = \boldsymbol{\mu}_F$

Note that for each $\boldsymbol{\mu}_M$ or $\boldsymbol{\mu}_F$ (a vector), there are three elements, i.e. $\boldsymbol{\mu}_M = \begin{bmatrix} \mu_{M,GABAA_diff1} \\ \mu_{M,GABAA_diff2} \\ \mu_{M,GABAA_diff3} \end{bmatrix}$.

The R code for profile analysis is as follow:

```
#Profile analysis , a series of hypothesis testings .
#To see if the profiles are parallel , coincide , horizontal .
library (profileR)
demo_prof<-pbg(demo_Y,demo_data$sexe , original.names=FALSE,profile.plot=FALSE)
summary(demo_prof)
```

The output is:

Hypothesis Tests: \$'Ho: Profiles are parallel'

	Multivariate Test	Statistic	Approx.F	num.df	den.df	p.value
1	Wilks	0.4201028	2.070554	2	3	0.2722911
2	Pillai	0.5798972	2.070554	2	3	0.2722911
3	Hotelling–Lawley	1.3803695	2.070554	2	3	0.2722911
4	Roy	1.3803695	2.070554	2	3	0.2722911

\$‘Ho: Profiles have equal levels ‘

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
group	1	0.011557	0.011557	19.56	0.0115 *
Residuals	4	0.002363	0.000591		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

\$‘Ho: Profiles are flat ‘

	F	df1	df2	p-value
1	1.311894	2	3	0.3896176

For H_0 : the 2 gender profiles are parallel, the p -value is 0.2722911, insignificant. We may conclude that male’s and female’s profiles are parallel.

For H_0 : the 2 gender profiles coincide, the p -value is 0.0115, significant. We may conclude that male’s profile is significantly different from female’s profile.

For H_0 : the 2 gender profiles are horizontal, the p -value is 0.3896176, insignificant. We may conclude that male’s and female’s profiles are horizontal.

We may also do an MANOVA test to test $H_0 : \mu_M = \mu_F$. The p -value is 0.007502, significant.

	Df	Pillai	approx F	num Df	den Df	Pr(>F)
demo_data\$sexe	1	0.99499	132.46	3	2	0.007502 **
Residuals	4					

We can also compute the contrast to see how different μ_M and μ_F are, although we already know that they are significantly different.

E.g. for variable GABAA_diff1 (the GABAA_diff at time 1), what is the difference between male and female? The contrast can be computed easily by SAS. But it seems that there is no package in R who does similar computation...The theory is there, but one has to write R code to compute the contrast.

Actually, the formula of the confidence interval of the contrast is stated in equation (4.3.3) on page 9 of

Chapter 4 (Multivariate Analysis of Variance). The general formula of the confidence interval is

$$\sum_{i=1}^k c_i \mathbf{a}' \bar{\mathbf{y}}_{i\cdot} \pm t_{\alpha/2, n-k} \sqrt{\frac{\mathbf{a}' \mathbf{E} \mathbf{a}}{n-k} \sum_{i=1}^k \frac{c_i^2}{n_i}},$$

where k is the number of group, $\bar{\mathbf{y}}_{i\cdot}$ is the vector of means for group i , and \mathbf{a} is a vector having the same length as $\bar{\mathbf{y}}_{i\cdot}$. \mathbf{E} is a matrix know as error sum of squares (detail can be omitted). In our example, there are two groups (gender), so $k = 2$. $\bar{\mathbf{y}}_{M\cdot}$ contains the means of GABAA_diff at 3 different time points for male, i.e.

$$\bar{\mathbf{y}}_{M\cdot} = \begin{bmatrix} \bar{y}_{M, \text{GABAA_diff at time 1}} \\ \bar{y}_{M, \text{GABAA_diff at time 2}} \\ \bar{y}_{M, \text{GABAA_diff at time 3}} \end{bmatrix}.$$

For instance, if we are interested in the confidence interval for the following difference

$$\bar{y}_{M, \text{GABAA_diff at time 1}} - \bar{y}_{F, \text{GABAA_diff at time 1}},$$

then we can set

$$\mathbf{a} = \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix}$$

$$c_1 = 1$$

$$c_2 = -1,$$

so that $\sum_{i=1}^k c_i \mathbf{a}' \bar{\mathbf{y}}_{i\cdot}$ becomes

$$\bar{y}_{M, \text{GABAA_diff at time 1}} - \bar{y}_{F, \text{GABAA_diff at time 1}}.$$

Similarly, if we set

$$\mathbf{a} = \begin{bmatrix} 0 \\ 1 \\ 0 \end{bmatrix}$$

$$c_1 = 1$$

$$c_2 = -1,$$

so that $\sum_{i=1}^k c_i \mathbf{a}' \bar{\mathbf{y}}_i$ becomes

$$\bar{y}_{M, \text{GABAA_diff at time 2}} - \bar{y}_{F, \text{GABAA_diff at time 2}}.$$

After writing the R code, the the confidence intervals are found. The codes are as follow:

```
#compute the confidence intervals of the difference of GABAA_diff1 between male and female
#set alpha (significance level) to be 0.05
a<-c(1,0,0)
colMeans_g1[1]-colMeans_g2[1]+qt(0.05/2,6-2)*sqrt(t(a)%*%E%*%a/(6-2)*2/3)
colMeans_g1[1]-colMeans_g2[1]+qt(1-0.05/2,6-2)*sqrt(t(a)%*%E%*%a/(6-2)*2/3)
#compute the confidence intervals of the difference of GABAA_diff2 between male and female
#set alpha (significance level) to be 0.05
a<-c(0,1,0)
colMeans_g1[2]-colMeans_g2[2]+qt(0.05/2,6-2)*sqrt(t(a)%*%E%*%a/(6-2)*2/3)
colMeans_g1[2]-colMeans_g2[2]+qt(1-0.05/2,6-2)*sqrt(t(a)%*%E%*%a/(6-2)*2/3)
#compute the confidence intervals of the difference of GABAA_diff3 between male and female
#set alpha (significance level) to be 0.05
a<-c(0,0,1)
colMeans_g1[3]-colMeans_g2[3]+qt(0.05/2,6-2)*sqrt(t(a)%*%E%*%a/(6-2)*2/3)
colMeans_g1[3]-colMeans_g2[3]+qt(1-0.05/2,6-2)*sqrt(t(a)%*%E%*%a/(6-2)*2/3)
```

For time 1 and time 2, as the upper confidence limits are negative, the GABAA_diff of male at time 1 and time 2 are all significantly smaller than GABAA_diff of female at time 1 and time 2. For time 3, the confidence interval contains zero, meaning that the difference between GABAA_diff of male at time 3 is not significantly different from GABAA_diff of female at time 3. It is consistent with the profile plot.

	Confidence interval
GABAA_diff1_male – GABAA_diff1_female	(−0.1546, −0.0854)
GABAA_diff2_male – GABAA_diff2_female	(−0.1930, −0.0070)
GABAA_diff3_male – GABAA_diff3_female	(−0.1574, 0.0707)

We can also compute the confidence interval for the following quantity:

$$\frac{1}{3} \sum_{i=1}^3 \text{GABAA_diff}_i_male - \frac{1}{3} \sum_{i=1}^3 \text{GABAA_diff}_i_female,$$

that is, the difference of average GABAA_diff over those 3 time points between male and female. The following code computes the confidence interval:

```
#compute the confidence intervals of difference of average GABAA_diff for those 3 time points
#set alpha (significance level) to be 0.05
a<-c(1/3,1/3,1/3)
1/3*(colMeans_g1[1]+colMeans_g1[2]+colMeans_g1[3]) - 1/3*(colMeans_g2[1]+colMeans_g2[2]+colMeans_g2[3])
1/3*(colMeans_g1[1]+colMeans_g1[2]+colMeans_g1[3]) - 1/3*(colMeans_g2[1]+colMeans_g2[2]+colMeans_g2[3])
```

The confidence interval is (−0.1429, −0.0327).

□

Example 10. Example 4.5 of the course Multivariate Data Analysis

Sepal length	Sepal width	Petal length	Petal width	Species
50	33	14	2	1
64	28	56	22	2
65	28	46	15	3
...

For sepal length, the 95% confidence limits are constructed...

Species comparison	Simultaneous 95% confidence intervals
3 - 2	(4.027, 9.013)
3 - 1	(13.327, 18.313)
2 - 3	(−9.013, −4.027)
2 - 1	(6.807, 11.793)
1 - 3	(−18.313, −13.327)
1 - 2	(−11.793, −6.807)

Confidence limits for other variables (sepal width, petal length and petal width) are also constructed.

□

Example 11. (Two way MANOVA) In this example, we have 8 participants and consider the effect of gender as well as the effect of age (young, old) on the GABAA_diff. The data set is as follows. Note that I added two more participants. In total, there are 8 participants.

Participant_id	gender	age	GABAA_diff at time 1	GABAA_diff at time 2	GABAA_diff at time 3
1	M	young	-0.19	-0.13	-0.21
2	F	young	-0.09	-0.07	-0.12
3	M	young	-0.23	-0.24	-0.17
4	F	young	-0.09	-0.09	-0.22
5	M	old	-0.20	-0.16	-0.18
6	F	old	-0.08	-0.07	-0.09
7	M	old	-0.22	-0.19	-0.21
8	F	old	-0.09	-0.1	-0.08

The following code conducts two-way multivariate ANOVA with interaction effect.

```
#conduct MANOVA
demo_manova <- manova(demo_Y ~ demo_data$sexe*demo_data$age, data=demo_data)
summary(demo_manova)
```

The output is as follow. It can be seen that the interaction effect between sexe and age is not significant. We can drop the interaction and estimate the model without interaction.

```

              Df  Pillai approx F num Df den Df    Pr(>F)
demo_data$sexe      1  0.99628   178.584      3      2 0.005574 **
demo_data$age        1  0.45134     0.548      3      2 0.696777
demo_data$sexe:demo_data$age  1  0.68626     1.458      3      2 0.431500
Residuals              4
— Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

R code for conducting multivariate ANOVA without interaction effect:

```
#conduct MANOVA without interaction
demo_manova <- manova(demo_Y ~ demo_data$sexe+demo_data$age, data=demo_data)
summary(demo_manova)
```

The output is as follow. The age does not have effect on GABAA_diff.

```

              Df  Pillai approx F num Df den Df    Pr(>F)
demo_data$sexe      1  0.99229   128.736      3      3 0.001146 **
```

```
demo_data$age    1 0.25195    0.337    3    3 0.802351
Residuals        5
— Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 1
```