

ST 437/537: Applied Multivariate and Longitudinal Data Analysis

Multiple Treatments Comparison

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Introduction

The ideas of the previous lecture [**One sample case**] (https://www.stat.ncsu.edu/people/maity/courses/st537-S2019/Lecture05_OneSample.html) can be used to develop procedures for comparison of two or more population mean vectors. We will first study such comparisons when the data arise from a paired design (measurements before/after), then when the data arises from multiple treatments.

Paired comparison

Key assumption

Each item/individual in the sample are measured twice: before and after a treatment/condition on the same attributes. Thus for individual i , we have a **pair** of vectors, X_i and Y_i , corresponding to measurements before and after treatment, where X_i and Y_i are $p \times 1$ vectors.

Suppose that X_1, \dots, X_n form a random sample with mean μ_1 , and similarly, Y_1, \dots, Y_n form a random sample with mean μ_2 . We want to answer the following questions:

- what are the possible values of $\mu_2 - \mu_1$?
- How to test $H_0: \mu_2 - \mu_1 = \delta_0$ vs. $H_0: \mu_2 - \mu_1 \neq \delta_0$ for some known vector δ_0 ?

As an illustrative example, consider [**the wastewater monitoring**] (**data/T6-1.dat**) (Table 6.1 in the Johnson and Wichern textbook). Municipal wastewater treatment plants are required by law to monitor their discharge into rivers and streams on a regular basis. Concerns about the reliability of data from one of these self-monitoring

programs led to a study in which samples of effluent were divided and sent to labs for testing. One half of each sample was sent to the Wisconsin State Laboratory of Hygiene and one half was sent to a private commercial laboratory routinely used in the monitoring program. Measurements of biochemical oxygen demand (BOD) and suspended solids (SS) were obtained for $n = 11$ sample splits from the two laboratories.

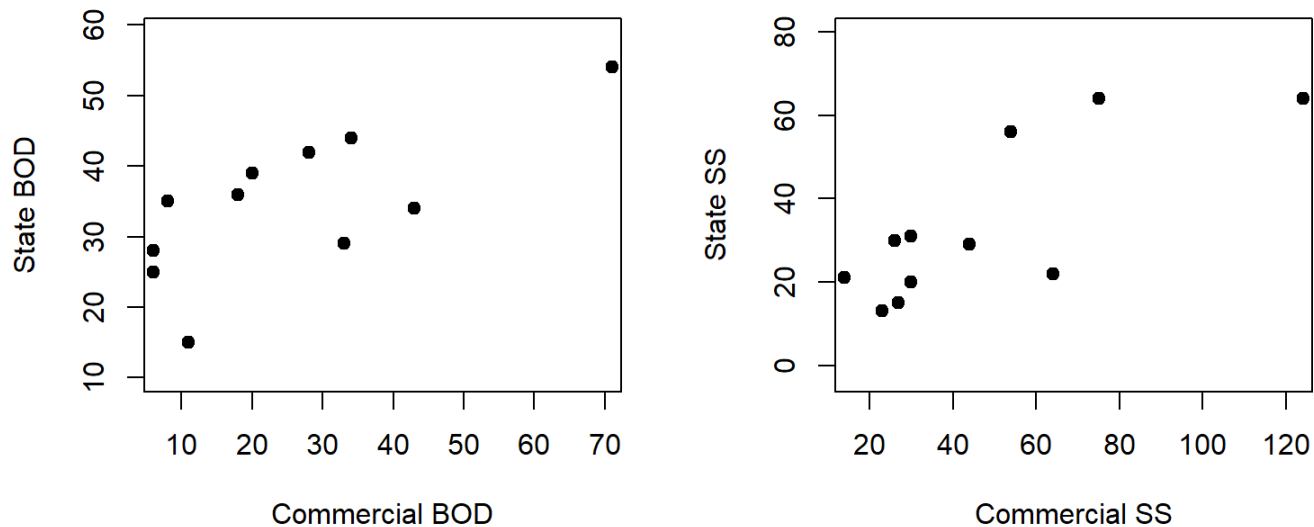
```
dat <- read.table("data/T6-1.dat")
colnames(dat) = c("Commercial_BOD", "Commercial_SS",
                  "StateLab_BOD", "StateLab_SS")
dat
```

```
##      Commercial_BOD Commercial_SS StateLab_BOD StateLab_SS
## 1                6          27          25          15
## 2                6          23          28          13
## 3               18          64          36          22
## 4                8          44          35          29
## 5               11          30          15          31
## 6               34          75          44          64
## 7               28          26          42          30
## 8               71         124          54          64
## 9               43          54          34          56
## 10              33          30          29          20
## 11              20          14          39          21
```

```
colMeans(dat)
```

```
## Commercial_BOD Commercial_SS StateLab_BOD StateLab_SS
##          25.27273          46.45455          34.63636          33.18182
```

```
library(MASS)
par(mfrow = c(1,2))
eqscplot(dat$Commercial_BOD, dat$StateLab_BOD, pch=19, xlab = "Commercial BOD", ylab =
  "State BOD")
eqscplot(dat$Commercial_SS, dat$StateLab_SS, pch=19, xlab = "Commercial SS", ylab =
  "State SS")
```



Our question of interest is whether the average (BOD, SS) measures are same between state and commercial labs.

Univariate case: paired t -test and intervals

Let us first review the univariate case. Specifically, for the i -th subject, we observe (X_i, Y_i) . Here $\mu_1 = E(X_i)$ and $\mu_2 = E(Y_i)$. We want to perform inference on $\mu_2 - \mu_1$.

We first define the difference $D_i = Y_i - X_i$. Note that

$$E(D_i) = \mu_2 - \mu_1.$$

We assume that $\{D_1, \dots, D_n\}$ is a random sample from a $N(\mu_2 - \mu_1, \sigma_D^2)$ distribution.

Thus a point estimator of $\mu_2 - \mu_1$ is the sample mean of differences, $\bar{D} = \sum_{i=1}^n D_i / n$. An point estimator of σ_D^2 is the sample variance of the differences, s_D^2 .

Hypothesis testing: To test $H_0: \mu_2 - \mu_1 = \delta_0$ vs. $H_0: \mu_2 - \mu_1 \neq \delta_0$ we use a one-sample t -test: define the test statistic

$$T = \frac{\bar{D} - \delta_0}{s_D / \sqrt{n}}.$$

Given a significance level α , we reject H_0 if

$$|T| \geq t_{n-1}(\alpha/2).$$

This test is often called a **paired t -test**.

Confidence interval: We can create a confidence interval for $\mu_2 - \mu_1$ using the t -distribution as well. Specifically, a $(1 - \alpha)100\%$ confidence interval for $\mu_2 - \mu_1$ is

$$\left(\bar{D} \pm t_{n-1}(\alpha/2) \frac{s_D}{\sqrt{n}} \right).$$

When the sample size is large ($n \geq 40$), we can obtain approximate/large sample z -interval and z -test by replacing $t_{n-1}(\alpha/2)$ by $z(\alpha/2)$.

Consider the watershade data, but only consider the variable BOD .

```
# extract the BOD variable for state and commercial labs
state_BOD <- dat$StateLab_BOD
com_BOD <- dat$Commercial_BOD

# Differences
D_BOD <- state_BOD - com_BOD

# 95% confidence Interval and 5% t-test
t.test(D_BOD)
```

```
##  
## One Sample t-test  
##  
## data: D_BOD  
## t = 2.2001, df = 10, p-value = 0.05243  
## alternative hypothesis: true mean is not equal to 0  
## 95 percent confidence interval:  
## -0.119457 18.846730  
## sample estimates:  
## mean of x  
## 9.363636
```

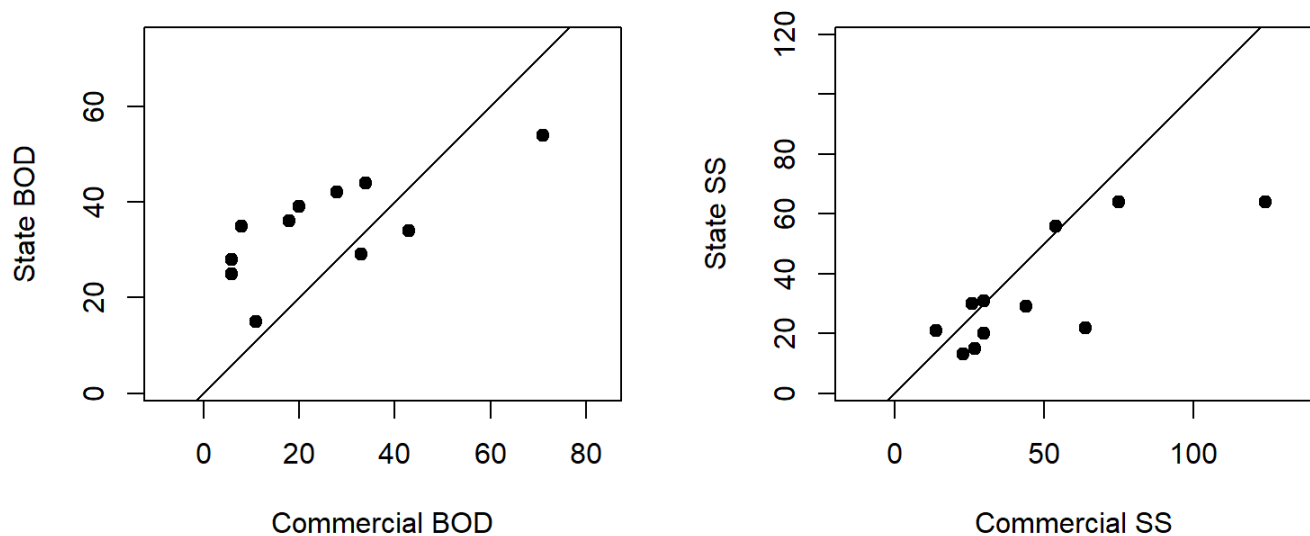
The 95% confidence interval is (- 0.119, 18.847). Also the p-value is larger than 0.05. We can conclude that the average BOD measurements of the state lab is the same as commercial labs.

We can run the same procedure on the `ss` variable; the results are show below.

```
##  
## One Sample t-test  
##  
## data: D_SS  
## t = -2.1515, df = 10, p-value = 0.05692  
## alternative hypothesis: true mean is not equal to 0  
## 95 percent confidence interval:  
## -27.0180504 0.4725958  
## sample estimates:  
## mean of x  
## -13.27273
```

It seems that for `ss` as well, we can conclude that the average SS measurements of the state labs is the same as commercial labs.

Looking at the results together, it seems state labs BOD measurements tend to be higher than that of commercil labs (95% CI captures mostly positive values). For `ss` , the situation is opposite: state labs SS measurements tend to be higher than that of commercil labs (95% CI captures mostly negative values). We display the data again, but using the same scale and a diagonal line.



Now let us consider the two variables (SS, BOD) together. The correlation matrix of the data is shown below.

```
round( cor(dat), 3)
```

```
##           Commercial_BOD Commercial_SS StateLab_BOD StateLab_SS
## Commercial_BOD           1.000         0.781         0.723         0.789
## Commercial_SS           0.781         1.000         0.677         0.790
## StateLab_BOD            0.723         0.677         1.000         0.604
## StateLab_SS            0.789         0.790         0.604         1.000
```

Notice the high correlation among the variables. It seems a multivariate analysis is well suited in this case.

Multivariate inference

In this situation, we first compute the **difference vector**

$$D_i = Y_i - X_i.$$

Note that $E(D_i) = \mu_2 - \mu_1$. We assume that the difference vectors $\{D_1, \dots, D_n\}$ is a random sample from a multivariate normal distribution $N(\mu_2 - \mu_1, \Sigma_D)$.

A point estimator of $\mu_2 - \mu_1$ is the sample mean vector \bar{D} ; we can also estimate Σ_D by the sample covariance matrix of the difference vectors, S_D .

Hypothesis testing: To test the null hypothesis $H_0: \mu_2 - \mu_1 = \delta_0$ vs

$H_1: \mu_2 - \mu_1 \neq \delta_0$, we calculate the **Hotelling's T^2 test** statistic

$$T^2 := \frac{(n-p)n}{(n-1)p} \bar{\mathbf{D}}^T \mathbf{S}_D^{-1} \bar{\mathbf{D}};$$

here $\bar{\mathbf{D}}$ and \mathbf{S}_D are the sample mean and sample covariance of d_i 's. If the null hypothesis is true then $T^2 \sim F_{p, n-p}$. Thus we reject H_0 at level α if the observed value of T^2 exceeds the critical value $F_{p, n-p}(\alpha)$.

Define δ_k to be the k -th component of $\mu_2 - \mu_1$. Also denote $\bar{\mathbf{D}} = (\bar{D}_1, \dots, \bar{D}_p)^T$.

Simultaneous confidence interval The $100(1 - \alpha)\%$ simultaneous confidence intervals of δ_k , $k = 1, \dots, p$ are:

$$\bar{D}_k \pm \sqrt{\frac{(n-1)p}{(n-p)} F_{p, n-p}(\alpha)} \sqrt{S_{D, kk}/n}, \quad k = 1, \dots, p;$$

here \bar{D}_k is the k -th element of the vector $\bar{\mathbf{D}}$, and $S_{D, kk}$ is the k th element of the diagonal of \mathbf{S}_D .

When the sample size is large, then $\frac{(n-1)p}{(n-p)} F_{p, n-p}(\alpha)$ is replaced by $\chi_p^2(\alpha)$.

The Bonferroni $100(1 - \alpha)\%$ simultaneous confidence intervals for δ_k 's are:

$$\bar{D}_k \pm t_{n-1} \left(\frac{\alpha}{2p} \right) \sqrt{S_{D, kk}/n}, \quad k = 1, \dots, p;$$

here $S_{D, kk}$ is the k th element of the diagonal of \mathbf{S}_D .

In the watershade data example, we first perform the Hotelling's T^2 test.

```

library(ICSNP)

# BOD differences
D_BOD <- dat$StateLab_BOD - dat$Commercial_BOD
# SS differences
D_SS <- dat$StateLab_SS - dat$Commercial_SS

# Obtain the vectors of difference between state and commercial
# Each ROW is one difference vector
Dmat <- cbind(D_BOD, D_SS)

# Hotelling's T2
HotellingsT2(Dmat)

```

```

##
## Hotelling's one sample T2-test
##
## data: Dmat
## T.2 = 6.1377, df1 = 2, df2 = 9, p-value = 0.02083
## alternative hypothesis: true location is not equal to c(0,0)

```

We can do this test manually as well.

```

n <- nrow(dat)
p <- 2

# Dbar, average of the observed differences
Dbar <- colMeans(Dmat)
# covariance
S_d <- cov(Dmat)
# T2
T2 <- (n - p)*n/(p*(n-1)) * t(Dbar) %*% solve(S_d) %*% Dbar
# critical value
crit <- qf(0.05, p, n-p, lower.tail = F)
# p-value
pv <- pf(c(T2), p, n-p, lower.tail = F)

data.frame(T2 = T2, critical = crit, pvalue = pv)

```

```

##          T2 critical      pvalue
## 1 6.13769 4.256495 0.02082779

```

From the results (p-value less than 0.05), we reject the null hypothesis that the means of the commercial and state lab measurements are same. Recall that we did not catch this difference using individual paired t-tests.

We compute the 95% simultaneous confidence for the mean differences below.


```
h <- sqrt((n-1)*p*crit/(n-p))* sqrt(diag(S_d)/n)
lower = Dbar - h
upper = Dbar + h
cbind(lower, upper)
```

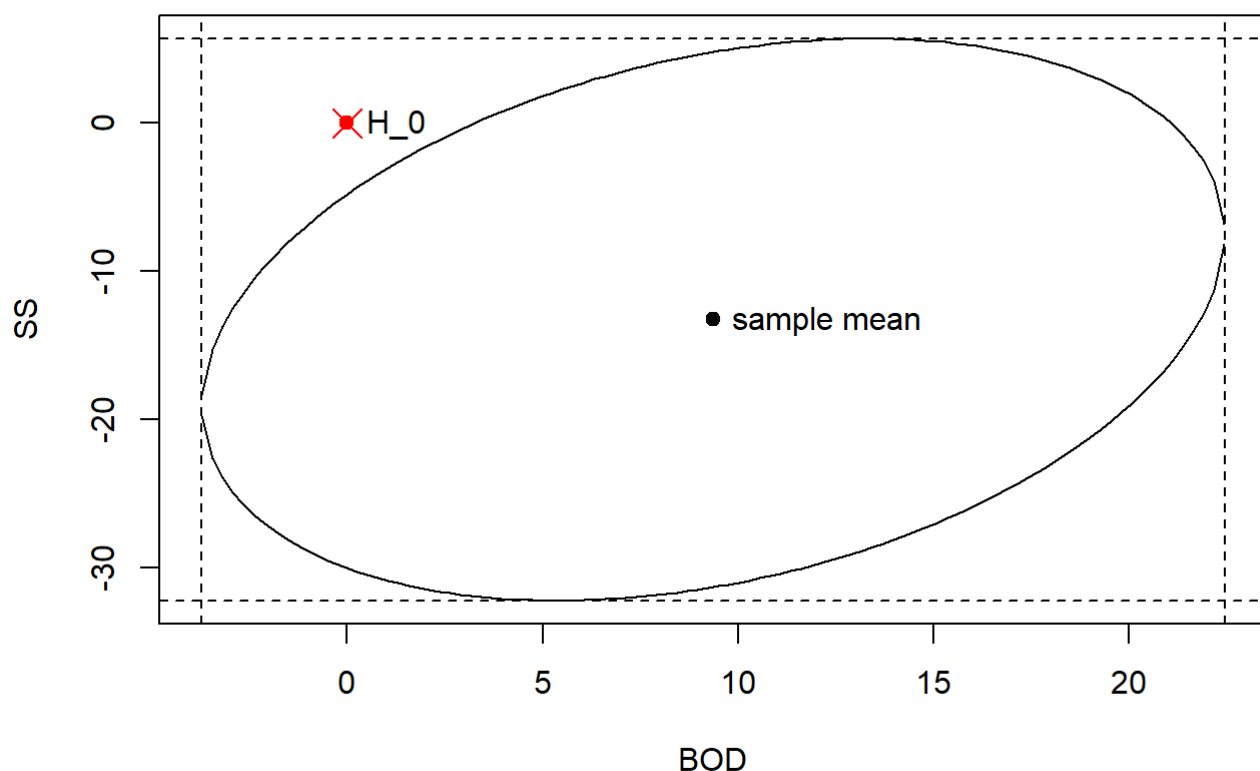
```
##           lower      upper
## D_BOD  -3.72600  22.453272
## D_SS   -32.24557   5.700119
```

Note that the simultaneous confidence intervals are wide, and as such they **should not be used for hypothesis testing**.

Geometry

The Hotelling's T^2 test essentially draws an ellipse (recall data ellipses in the multivariate normal lecture) around the sample mean and checks whether the proposed value (the value proposed in H_0) lies in the ellipse or not. We reject H_0 if the proposed value does not fall inside the ellipse. This ellipse is often referred to as a *confidence region*.

For our dataset, the ellipse is shown below. We can see that the null value (0, 0) (marked by the red x) falls outside the confidence region. This matches with the result we obtained from the hypothesis test, where we rejected $H_0: \mu_2 - \mu_1 = 0$.



The vertical and horizontal lines representing the projection of the ellipse onto the x- and y-axis are in fact the corresponding simultaneous confidence intervals we constructed before.

It is interesting to see that the simultaneous intervals do contain zero. This is because the simultaneous confidence coefficient applies to the entire set of intervals that could be constructed for all possible linear combinations of the form $a_1\delta_1 + a_2\delta_2$, where δ_1 is the mean difference in SS and δ_2 is the mean difference in BOD. The particular intervals obtained using $(a_1 = 1, a_2 = 0)$ and $(a_1 = 0, a_2 = 1)$ contain zero. However, there are other choices of a_1 and a_2 that will produce intervals not containing zero.

In other words, if we were to not reject $H_0: \delta = 0$, then **all** simultaneous intervals (corresponding to all possible choices of a_1 and a_2) would contain zero.

To see an example (purely for demonstration, not interpretation), consider the linear combination $1.5\delta_1 - 0.5\delta_2$. A point estimator of this parameter is $1.5\bar{D}_1 - 0.5\bar{D}_2$, and the variance can be estimated by $(1.5, -0.5)\mathbf{S}_D(1.5, -0.5)^T/n$. The 95% simultaneous confidence interval for this particular choice of $a_1 = 1.5$ and $a_2 = -0.5$ is shown below.

```
## [1] 1.666067 39.697570
```

Clearly, for this choice of a_1 and a_2 , the interval does not contain zero. If H_0 was indeed true, every interval (that is, for all choices for a_1 and a_2) would contain zero.

Multiple treatments comparison

Let us consider the case where each sampling unit is subjected to more than two treatments. In this course, we will only consider the case where only one variable is measured for each subject under each treatment.

As an illustrative example, we consider the **[anesthesia dataset] (data/T6-2.dat)** in Table 6.2 in the Johnson and Wichern textbook. The dataset measures anesthetizing effect of CO_2 and halothane in dogs.

“Improved anesthetics are often developed by first studying their effects on animals. In one study 19 dogs were initially given the drug pentobarbitol. Each dog was then administered carbon dioxide CO_2 at each of two pressure levels. Next, halothane (H) was added and then administration of CO_2 was repeated. The response, milliseconds between heartbeats was measured for the four treatment combinations.”

The data are displayed in Table 6.2 in the Johnson and Wichern textbook. The treatment combinations are shown below.

Treatment	CO ₂ pressure	Halothane
1	high	present
2	low	present
3	high	absent
4	low	absent

```
dat <- as.matrix( read.table("data/T6-2.dat") )
colnames(dat) = c("trt 1", "trt 2", "trt 3", "trt 4")
head(dat)
```

```
##      trt 1 trt 2 trt 3 trt 4
## [1,]   426   609   556   600
## [2,]   253   236   392   395
## [3,]   359   433   349   357
## [4,]   432   431   522   600
## [5,]   405   426   513   513
## [6,]   324   438   507   539
```

Define X_{ij} = the response for the i -th dog under j -th treatment. We define

$$\mu_j = \text{mean response under } j\text{-th treatment} = E(X_{ij}).$$

We might be interested in testing the following hypotheses:

1. Null effect/no treatment effect: $H_0: \mu_1 = \mu_2 = \mu_3 = \mu_4$

or equivalently, testing the following jointly:

2. No effect of halothane. $H_0: (\mu_1 + \mu_2) - (\mu_3 + \mu_4) = 0$
3. No effect of CO₂. $H_0: (\mu_1 + \mu_3) - (\mu_2 + \mu_4) = 0$
4. No interaction of CO₂ and halothane. $H_0: (\mu_1 + \mu_4) - (\mu_2 + \mu_3) = 0$

In general, all the hypotheses presented above can be written as

$$H_0: C\boldsymbol{\mu} = 0,$$

where C is $q \times 4$ matrix of contrasts. For example, the first hypothesis (1.) of no treatment effect $H_0: \mu_1 = \mu_2 = \mu_3 = \mu_4$ can be written as

$H_0: \mu_1 - \mu_2 = 0, \mu_1 - \mu_3 = 0, \mu_1 - \mu_4 = 0$. Thus we can write H_0 as

$$\underbrace{\begin{bmatrix} 1 & -1 & 0 & 0 \\ 1 & 0 & -1 & 0 \\ 1 & 0 & 0 & -1 \end{bmatrix}}_{\mathbf{C}} \underbrace{\begin{pmatrix} \mu_1 \\ \mu_2 \\ \mu_3 \\ \mu_4 \end{pmatrix}}_{\boldsymbol{\mu}} = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

Similarly, for the hypotheses in (2. – 4.) can be written as

$$\underbrace{\begin{bmatrix} 1 & 1 & -1 & -1 \\ 1 & -1 & 1 & -1 \\ 1 & -1 & -1 & 1 \end{bmatrix}}_{\mathbf{C}} \underbrace{\begin{pmatrix} \mu_1 \\ \mu_2 \\ \mu_3 \\ \mu_4 \end{pmatrix}}_{\boldsymbol{\mu}} = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

Let us collect the data for the i -th dog (in general subject/item) as $\mathbf{X}_i = (X_{i1}, \dots, X_{i4})^T$. Thus $\boldsymbol{\mu} = E(\mathbf{X}_i)$. We know that a point estimator of $\boldsymbol{\mu}$ is $\bar{\mathbf{X}}$ and a point estimator of $\boldsymbol{\Sigma} = \text{cov}(\mathbf{X})$ is the sample covariance matrix \mathbf{S} . Hence we have

Point estimator of $\mathbf{C}\boldsymbol{\mu}$ is $\mathbf{C}\bar{\mathbf{X}}$

Covariance matrix of $\mathbf{C}\bar{\mathbf{X}}$ is \mathbf{CSC}^T/n

Hotelling's T^2 test: Testing $H_0: \mathbf{C}\boldsymbol{\mu} = 0$ is done using the Hotelling's T^2 test statistic:

$$T^2 := \frac{(n-q)n}{(n-1)q} (\mathbf{C}\bar{\mathbf{X}})^T (\mathbf{CSC}^T)^{-1} \mathbf{C}\bar{\mathbf{X}};$$

here $\bar{\mathbf{X}}$ and \mathbf{S} are the sample mean and covariance of \mathbf{X}_i 's. If the null hypothesis is true then $T^2 \sim F_{q, n-q}$. Thus we reject H_0 at level α if the observed value of T^2 exceeds the critical value $F_{q, n-q}(\alpha)$.

Note: in the formula above, q denotes the number of rows/contrasts in C that are being tested.

Let us perform the hypothesis testing for the dataset at hand. We start with the equality of the treatment means $H_0: \mu_1 = \dots = \mu_4$. Recall the contrast matrix for this hypothesis is

$$C = \begin{bmatrix} 1 & -1 & 0 & 0 \\ 1 & 0 & -1 & 0 \\ 1 & 0 & 0 & -1 \end{bmatrix}$$

A function for testing multiple contrasts is shown below.

```
T2.contrast <- function(data.matrix, contrast.matrix, alpha = 0.05){
  # Input args
  # data.matrix: n x p matrix, each row is one subject, each col is one treatment
  # contrast.matrix: q x p matrix C, each row is one contrast
  # alpha: significance level, default 0.05

  dat <- data.matrix
  C <- contrast.matrix

  # sample mean vector
  xbar <- colMeans(dat)
  # sample covariance matrix
  S <- cov(dat)

  # parameters
  n <- nrow(dat)
  q <- nrow(C)

  # Intermediate quantities
  invCSC <- solve(C%*%S %*% (t(C)))
  Cxbar <- C %*% xbar
  # test statistic
  T2 <- n*(n-q)/((n-1)*q) * (t(Cxbar)) %*% invCSC %*% (Cxbar)
  # critical value
  critical_value_F = qf(p = 0.05, df1 = q, df2 = n-q, lower.tail = F)
  # p-value
  pv <- pf(T2, df1 = q, df2 = n-q, lower.tail = F)

  # display the results
  results <- data.frame(T2 = T2, critical = critical_value_F,
                        df1 = q, df2 = n-q, pvalue = pv)

  return(results)
}
```

Let us use this function to test the hypothesis of equality of mean.

```
# contrast matrix for testing equality of means
C <- cbind(c(1,1,1), -diag(1, 3))
C
```

```
##      [,1] [,2] [,3] [,4]
## [1,]    1   -1    0    0
## [2,]    1    0   -1    0
## [3,]    1    0    0   -1
```

```
# Test
T2.contrast(dat, C)
```

```
##      T2 critical df1 df2      pvalue
## 1 34.37521 3.238872   3  16 3.317767e-07
```

Clearly, there is strong evidence that the four treatment means are not equal.

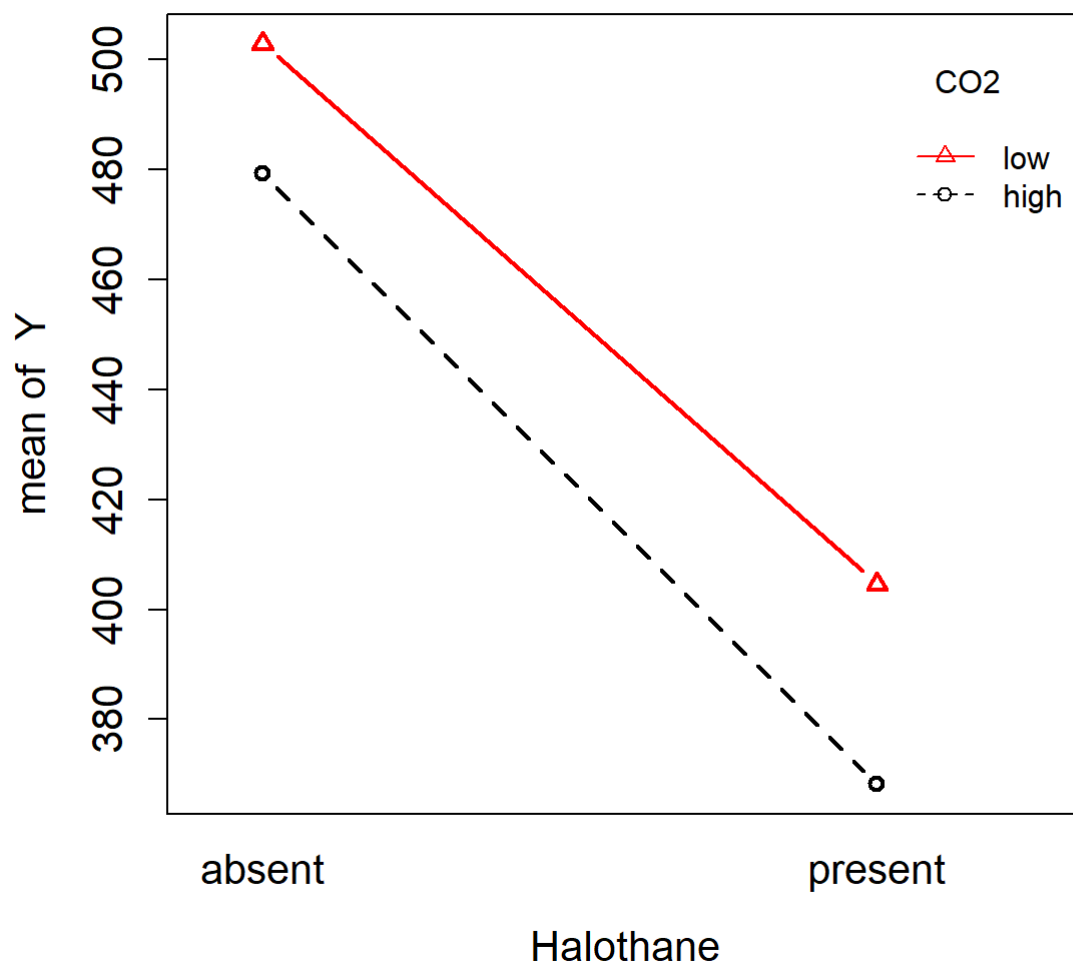
To investigate further, we can test individual hypotheses 2. – 4. above to identify the interaction and main effects of halothane and CO₂. We first start with testing the interaction. Let us look at the interaction plot. We can use the function `interaction.plot()` to do this.

```
# vectorize the data for interaction plot
# this creates a vector by concatenating columns
Y = c(dat)

# Define the treatment combinations
CO2 = c(rep("high", 19), rep("low", 19), rep("high", 19), rep("low", 19))
Halothane = c(rep("present", 19), rep("present", 19), rep("absent", 19), rep("absent", 19))

# interaction plot
interaction.plot(Halothane, CO2, Y, lwd=2, col=c(1,2), cex.axis=1.3, cex.lab=1.3, main="Interaction plot", cex.main=2, type="b", pch=1:2)
```

Interaction plot



Let us formally test the hypothesis of no interaction, $H_0: (\mu_1 + \mu_4) - (\mu_2 + \mu_3) = 0$

```
# contrast for no interaction
C <- matrix(c(1, -1, -1, 1), nrow = 1)

# test
T2.contrast(dat, C)
```

```
##          T2 critical df1 df2    pvalue
## 1 0.4112318 4.413873   1  18 0.5294265
```

It is evident that there is no interaction effect. We can similarly test for the main effects of halothane and CO2.

Simultaneous confidence intervals

We can also create simultaneous intervals for any set of given contrasts $c_1^T \mu, \dots, c_k^T \mu$ (you can think of c_1, \dots, c_k as rows of C).

The $100(1 - \alpha)\%$ simultaneous confidence intervals are

$$\mathbf{c}^T \bar{\mathbf{x}} \pm \sqrt{\frac{(n-1)q}{n-q} F_{q, n-q}(\alpha)} \sqrt{\frac{\mathbf{c}^T \mathbf{S} \mathbf{c}}{n}}$$

will contain $\mathbf{c}^T \boldsymbol{\mu}$ simultaneously for **all** \mathbf{c} with probability $1 - \alpha$.

In our data set, let us create 95% simultaneous confidence intervals for

- effect of halothane: $(\mu_1 + \mu_2) - (\mu_3 + \mu_4)$
- effect of CO₂: $(\mu_1 + \mu_3) - (\mu_2 + \mu_4)$
- interaction of CO₂ and halothane: $(\mu_1 + \mu_4) - (\mu_2 + \mu_3)$.

As mentioned before, the contrast matrix is

$$\mathbf{C} = \begin{bmatrix} 1 & 1 & -1 & -1 \\ 1 & -1 & 1 & -1 \\ 1 & -1 & -1 & 1 \end{bmatrix}$$

```
# contrast matrix
C <- matrix(c(1, 1, -1, -1, 1, -1, 1, -1, -1, -1, 1), ncol=4, byrow=T)
rownames(C) <- c("Halothane (present - absent)", "CO2 (high - low)", "H-CO2 Interaction")
p <- ncol(C) # number of variables
q <- nrow(C) # number of contrasts
n <- nrow(dat) # sample size
C
```

```
##           [,1] [,2] [,3] [,4]
## Halothane (present - absent)  1    1   -1   -1
## CO2 (high - low)             1   -1    1   -1
## H-CO2 Interaction            1   -1   -1    1
```



```

# sample mean vector
xbar = colMeans(dat)

# sample covariance matrix
S <- cov(dat)

# compute C xbar
mid <- C %*% xbar

# compute (C S C^T)
M <- C %*% S %*% t(C)

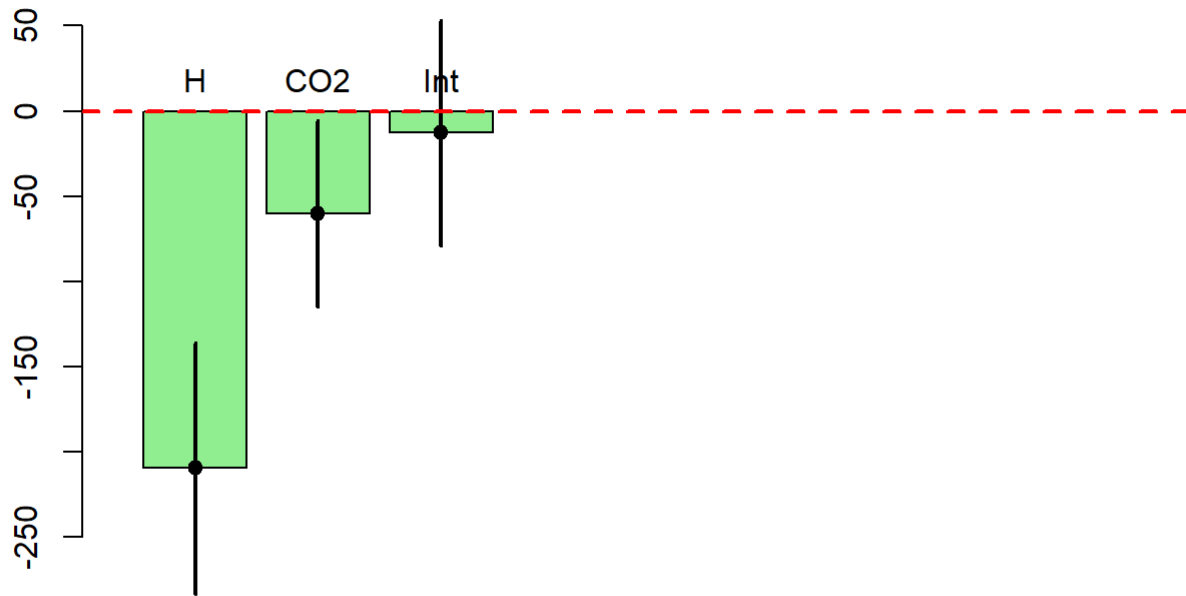
# critical value (scaled)
critical_value_F = sqrt(
  ( (n-1)*q/(n-q) ) * qf(p = 0.05, df1 = q, df2 = n-q, lower.tail = F)
)
# std errors
sdvec <- sqrt(diag(M)/n)

# Intervals with point estimates
result <- data.frame(Estimate = mid,
                     Lower = mid - critical_value_F*sdvec,
                     Upper = mid + critical_value_F*sdvec)
result

```

##	Estimate	Lower	Upper
## Halothane (present - absent)	-209.31579	-282.98128	-135.65030
## CO2 (high - low)	-60.05263	-114.72708	-5.37818
## H-CO2 Interaction	-12.78947	-78.72858	53.14964

While there is no interaction between halothane and CO2 effects, it seems the main effects of CO2 and Halothane are statistically significant. It seems that absence of halothane is associated with higher mean response, and high level of CO2 with lower mean response.



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