

What is paired samples t-test?

The **paired samples t-test** is used to compare the means between two related groups of samples. In this case, you have two values (i.e., pair of values) for the same samples. This article describes how to compute **paired samples t-test** using **R software**.

As an example of data, 20 mice received a treatment X during 3 months. We want to know whether the treatment X has an impact on the weight of the mice.

To answer to this question, the weight of the 20 mice has been measured before and after the treatment. This gives us 20 sets of values before treatment and 20 sets of values after treatment from measuring twice the weight of the **same mice**.

In such situations, **paired t-test** can be used to compare the mean weights before and after treatment.

Paired t-test analysis is performed as follow:

1. Calculate the difference (d) between each pair of value
2. Compute the mean (m) and the standard deviation (s) of d
3. Compare the average difference to 0. If there is any significant difference between the two pairs of samples, then the mean of d (m) is expected to be far from 0.

Paired t-test can be used only when the difference d is normally distributed. This can be checked using **Shapiro-Wilk test**.

Research questions and statistical hypotheses

Typical research questions are:

- ?
- 1. whether the mean difference (m) is equal to 0?
2. whether the mean difference (m) is less than 0?
3. whether the mean difference (m) is greater than 0?

In statistics, we can define the corresponding *null hypothesis* (H_0) as follow:

1. $H_0 : m = 0$
2. $H_0 : m \leq 0$
3. $H_0 : m \geq 0$

The corresponding *alternative hypotheses* (H_a) are as follow:

1. $H_a : m \neq 0$ (different)
2. $H_a : m > 0$ (greater)
3. $H_a : m < 0$ (less)

!

Note that:

- Hypotheses 1) are called **two-tailed tests**
- Hypotheses 2) and 3) are called **one-tailed tests**

Formula of paired samples t-test

t-test statistic value can be calculated using the following formula:

$$t = \frac{m}{s/\sqrt{n}}$$

where,

- **m** is the mean differences
- **n** is the sample size (i.e., size of d).
- **s** is the standard deviation of d

We can compute the p-value corresponding to the absolute value of the **t-test statistics** ($|t|$) for the **degrees of freedom** (df): $df = n - 1$.

✓ If the p-value is inferior or equal to 0.05, we can conclude that the difference between the two paired samples are significantly different.

Visualize your data and compute paired t-test in R

R function to compute paired t-test

To perform paired samples t-test comparing the means of two paired samples (x & y), the R function **t.test()** can be used as follow:

```
t.test(x, y, paired = TRUE, alternative = "two.sided")
```

- **x,y**: numeric vectors
- **paired**: a logical value specifying that we want to compute a paired t-test
- **alternative**: the alternative hypothesis. Allowed value is one of “two.sided” (default), “greater” or “less”.

Import your data into R

Here, we'll use an example data set, which contains the weight of 10 mice before and after the treatment.

```
# Data in two numeric vectors
# ++++++
# Weight of the mice before treatment
before <-c(200.1, 190.9, 192.7, 213, 241.4, 196.9, 172.2, 185.5, 205.2, 193.7)
# Weight of the mice after treatment
after <-c(392.9, 393.2, 345.1, 393, 434, 427.9, 422, 383.9, 392.3, 352.2)
# Create a data frame
my_data <- data.frame(
  group = rep(c("before", "after"), each = 10),
  weight = c(before, after)
)
```

?

We want to know, if there is any significant difference in the mean weights after treatment?

Check your data

```
# Print all data
print(my_data)

  group weight
1 before 200.1
2 before 190.9
3 before 192.7
4 before 213.0
5 before 241.4
6 before 196.9
```

```
8 before 185.5
9 before 205.2
10 before 193.7
11 after 392.9
12 after 393.2
13 after 345.1
14 after 393.0
15 after 434.0
16 after 427.9
17 after 422.0
18 after 383.9
19 after 392.3
20 after 352.2
```

Compute summary statistics (mean and sd) by groups using the dplyr package.

- To install **dplyr** package, type this:

```
install.packages("dplyr")
```

- Compute summary statistics by groups:

```
library("dplyr")
group_by(my_data, group) %>%
  summarise(
    count = n(),
    mean = mean(weight, na.rm = TRUE),
    sd = sd(weight, na.rm = TRUE)
)
```

```
Source: local data frame [2 x 4]
  group count   mean      sd
  (fctr) (int)  (dbl)  (dbl)
1  after    10 393.65 29.39801
2 before     10 199.16 18.47354
```

Visualize your data using box plots

Install the latest version of ggpublisher from GitHub as follow (recommended):

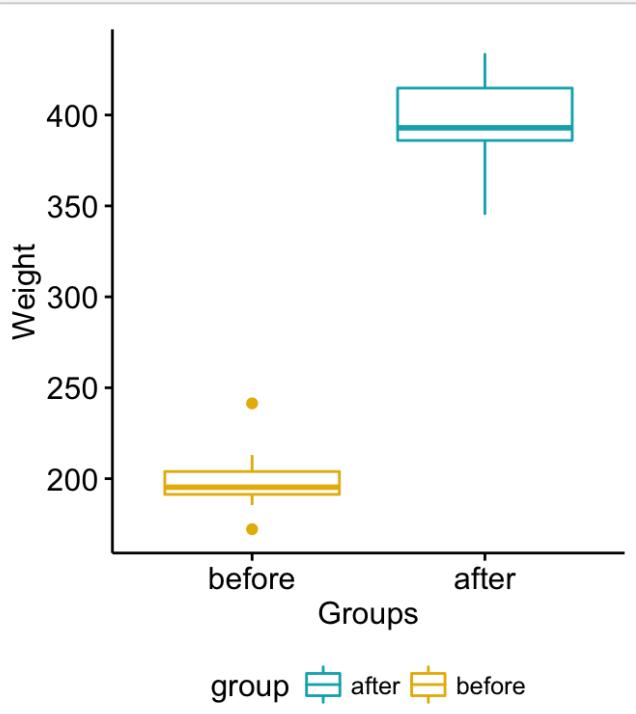
```
# Install
if(!require(devtools)) install.packages("devtools")
devtools::install_github("kassambara/ggpublisher")
```

- Or, install from CRAN as follow:

```
install.packages("ggpubr")
```

- Visualize your data:

```
# Plot weight by group and color by group
library("ggpubr")
ggboxplot(my_data, x = "group", y = "weight",
          color = "group", palette = c("#00AFBB", "#E7B800"),
          order = c("before", "after"),
          ylab = "Weight", xlab = "Groups")
```



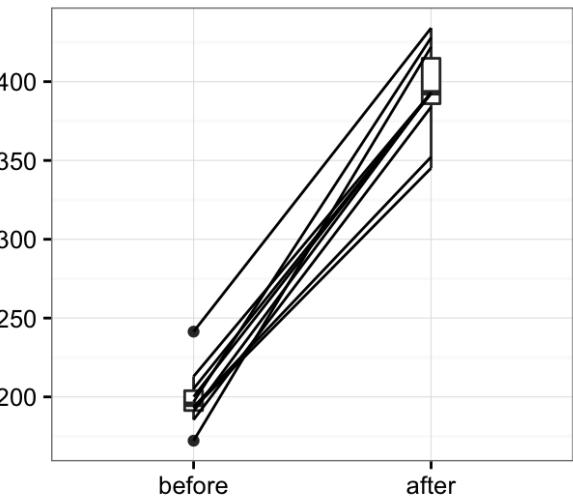
⚠️ Box plots show you the increase, but lose the paired information. You can use the function **plot.paired()** [in **PairedData** package] to plot paired data ("before - after" plot).

- Install pairedData package:

```
install.packages("PairedData")
```

- Plot paired data:

```
# Subset weight data before treatment
before <- subset(my_data, group == "before", weight,
                  drop = TRUE)
# subset weight data after treatment
after <- subset(my_data, group == "after", weight,
                  drop = TRUE)
# Plot paired data
library(PairedData)
pd <- paired(before, after)
plot(pd, type = "profile") + theme_bw()
```



Preleminary test to check paired t-test assumptions

? Assumption 1: Are the two samples paired?

Yes, since the data have been collected from measuring twice the weight of the same mice.

? Assumption 2: Is this a large sample?

No, because $n < 30$. Since the sample size is not large enough (less than 30), we need to check whether the differences of the pairs follow a normal distribution.

? How to check the normality?

Use Shapiro-Wilk normality test

- Null hypothesis: the data are normally distributed
- Alternative hypothesis: the data are not normally distributed

```
# compute the difference
d <- with(my_data,
           weight[group == "before"] - weight[group == "after"])
# Shapiro-Wilk normality test for the differences
shapiro.test(d) # => p-value = 0.6141
```

✓ From the output, the p-value is greater than the significance level 0.05 implying that the distribution of the differences (d) are not significantly different from normal distribution. In other words, we can assume the normality.

Compute paired samples t-test

?

Question : Is there any significant changes in the weights of mice after treatment?

1) Compute paired t-test - Method 1:

The data are saved in two different numeric vectors.

```
# Compute t-test
res <- t.test(before, after, paired = TRUE)
res
```

```
Paired t-test
data: before and after
t = -20.883, df = 9, p-value = 6.2e-09
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-215.5581 -173.4219
sample estimates:
mean of the differences
-194.49
```

2) Compute paired t-test - Method 2:

The data are saved in a data frame.

```
# Compute t-test
res <- t.test(weight ~ group, data = my_data, paired = TRUE)
res
```

```
Paired t-test
data: weight by group
t = 20.883, df = 9, p-value = 6.2e-09
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
173.4219 215.5581
sample estimates:
mean of the differences
194.49
```

! As you can see, the two methods give the same results.

In the result above :

- **t** is the **t-test statistic** value ($t = 20.88$),
- **df** is the degrees of freedom ($df = 9$),
- **p-value** is the significance level of the **t-test** ($p\text{-value} = 6.210^{-9}$).
- **conf.int** is the **confidence interval** ($\text{conf.int} = [173.42, 215.56]$)
- **sample estimates** is the mean differences between pairs ($\text{mean} = 194.49$).

! Note that:

- if you want to test whether the average weight before treatment is less than the average weight after treatment, type this:

```
t.test(weight ~ group, data = my_data, paired = TRUE,
       alternative = "less")
```

- Or, if you want to test whether the average weight before treatment is greater than the average weight after treatment, type this

```
t.test(weight ~ group, data = my_data, paired = TRUE,
       alternative = "greater")
```

Interpretation of the result

 The **p-value** of the test is 6.210^{-9} , which is less than the significance level $\alpha = 0.05$. We can then reject null hypothesis and conclude that the average weight of the mice before treatment is significantly different from the average weight after treatment with a **p-value** = 6.210^{-9} .

The format of the **R** code to use for getting these values is as follow:

```
# printing the p-value
res$p.value
```

```
[1] 6.200298e-09
```

```
# printing the mean
res$estimate
```

```
mean of the differences
194.49
```

```
# printing the confidence interval
res$conf.int
```

```
[1] 173.4219 215.5
attr(,"conf.level")
[1] 0
```