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Z MBP

Biostatistics

Microbiome: a Hands-on Experience 2024

Center for Plant Molecular Biology - ZMBP

Microbiome: a Hands-on Experience

Agenda:

- **Intro to Statistics**
- **Getting to know the data**
- **Data handling and visualization in R**
- **Hypothesis Testing**

Intro to Statistics

Intro to Statistics

Biostatistics or medical biometry:

- a branch of statistics that applies statistical techniques and principles to scientific research to a wide range of topics in health-related fields
  - e.g. medicine, biology, and public health
- the development of new tools to study these areas.
- includes the design of biological experiments, the collection and analysis of data from those experiments, and the interpretation of the results.

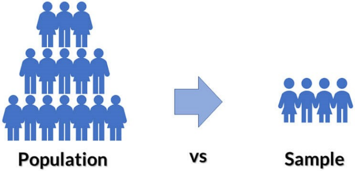
Population vs. Sample

Population: is the set of all individuals of interest

- often large and impossible to obtain measurements from all individuals
- Examples: all individuals with Migraine, all people who take a daily vitamin supplement in Germany, ...

Sample: a set of individuals selected from a population

- representative and generalizable



Parameter vs. Statistic

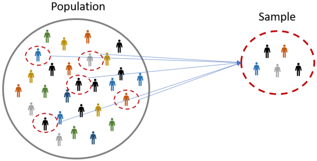
Parameter: describes a population

Statistic: describes a sample

A statistic is used to estimate a parameter

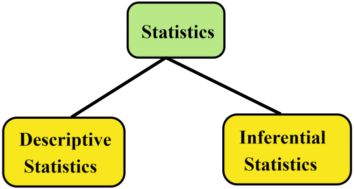
For example:

- μ describes the population mean
- $\bar{X}$ represents the sample mean (average)



Intro to Statistics

Descriptive vs. Inferential



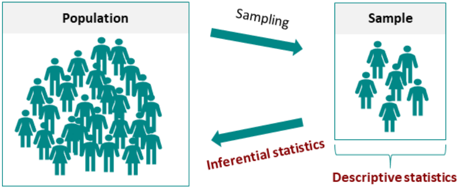
Descriptive statistics methods: are used to collect, summarize, organize, simplify, and describe data.

- Examples: mean, median, variance, standard deviation

Descriptive vs. Inferential

Inferential Statistics methods: concluding and making decisions concerning a population based only on a sample

- Examples: regression analysis, confidence interval, and hypothesis testing



Sampling error

- arises when a sample does not represent the whole population.
- the discrepancy between a sample statistic and the true population parameter
- Increasing the sample size and random selection can reduce the errors.
- Example:

true population value: $\mu = 19.5$
sample statistic: $\bar{X} = 18$
sampling error: $\mu - \bar{X} = 1.5$

there is a discrepancy of 1.5

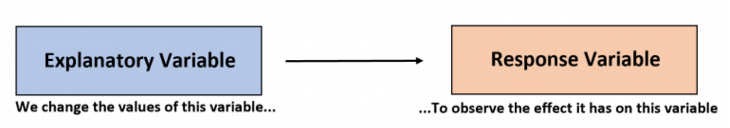
Intro to Statistics

Types of Variables

Independent (Explanatory): is the variables that can be altered or manipulated in research (e.g., caffeine dose)

Dependent (Response): is the result of manipulation done to the variables (e.g., reaction times).

Example: X and Y in linear regression model $Y = AX + \epsilon$



Explanatory Variable

Response Variable

We change the values of this variable...

...To observe the effect it has on this variable

Getting to know the data

Getting started with data

GREIN:

GEO RNA-seq Experiments Interactive Navigator is an Interactive Web Platform for Re-analyzing GEO RNA-seq data and the large number $(> 6,000)$ of already processed datasets Mahi et al. [2019].

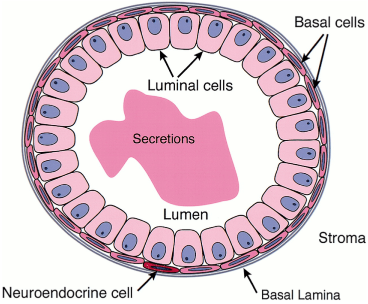
Link: `http://www.ilincs.org/apps/grein/`

GREIN provides both raw and normalized counts (normalized for differences in sequencing depth and composition bias).

Getting started with data

Data set: GEO code GSE60450.

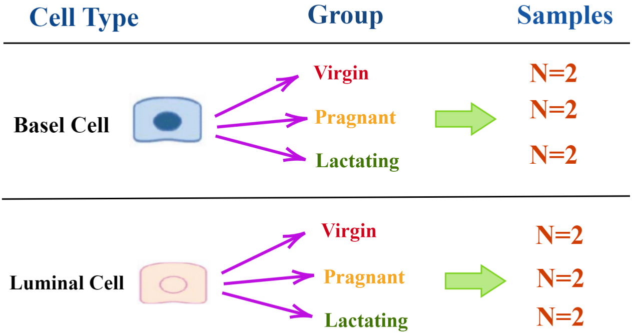
- RNA-seq data from the paper by Fu et al. [2015]
- examined expression in basal and luminal cells from mice at different stages (virgin, pregnant, and lactating



Getting started with data

Data set: GEO code GSE60450

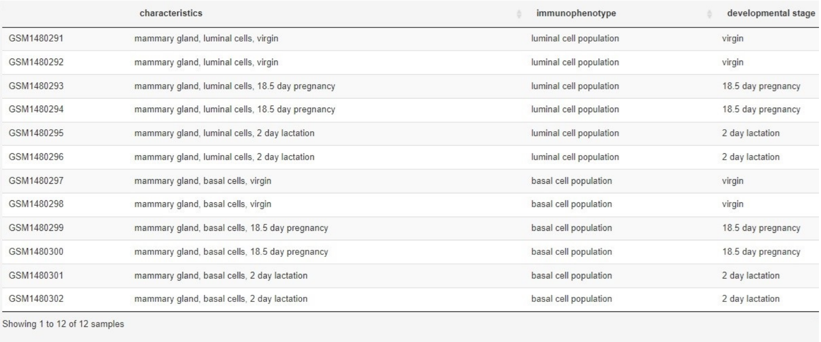
- There are 6 groups and 2 samples per group, 12 samples in total.



Cell Type	Group	Samples
Basel Cell	Virgin	N=2
	Pragnant	N=2
	Lactating	N=2
Luminal Cell	Virgin	N=2
	Pragnant	N=2
	Lactating	N=2

Getting to know the data

Metadata:

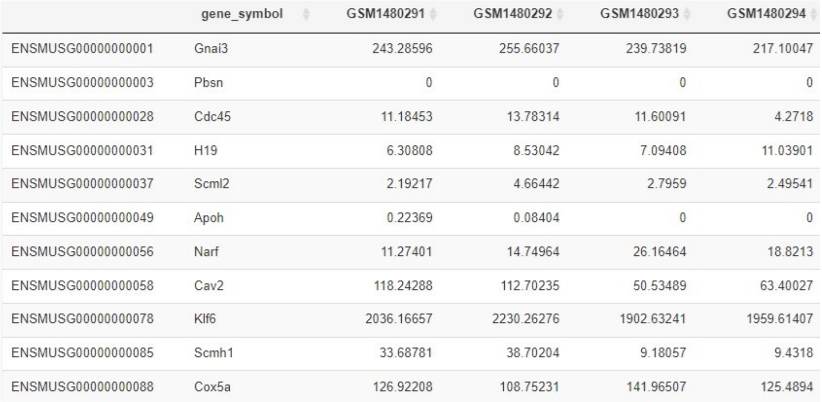


	characteristics	immunophenotype	developmental stage
GSM1480291	mammary gland, luminal cells, virgin	luminal cell population	virgin
GSM1480292	mammary gland, luminal cells, virgin	luminal cell population	virgin
GSM1480293	mammary gland, luminal cells, 18.5 day pregnancy	luminal cell population	18.5 day pregnancy
GSM1480294	mammary gland, luminal cells, 18.5 day pregnancy	luminal cell population	18.5 day pregnancy
GSM1480295	mammary gland, luminal cells, 2 day lactation	luminal cell population	2 day lactation
GSM1480296	mammary gland, luminal cells, 2 day lactation	luminal cell population	2 day lactation
GSM1480297	mammary gland, basal cells, virgin	basal cell population	virgin
GSM1480298	mammary gland, basal cells, virgin	basal cell population	virgin
GSM1480299	mammary gland, basal cells, 18.5 day pregnancy	basal cell population	18.5 day pregnancy
GSM1480300	mammary gland, basal cells, 18.5 day pregnancy	basal cell population	18.5 day pregnancy
GSM1480301	mammary gland, basal cells, 2 day lactation	basal cell population	2 day lactation
GSM1480302	mammary gland, basal cells, 2 day lactation	basal cell population	2 day lactation

Showing 1 to 12 of 12 samples

Getting to know the data

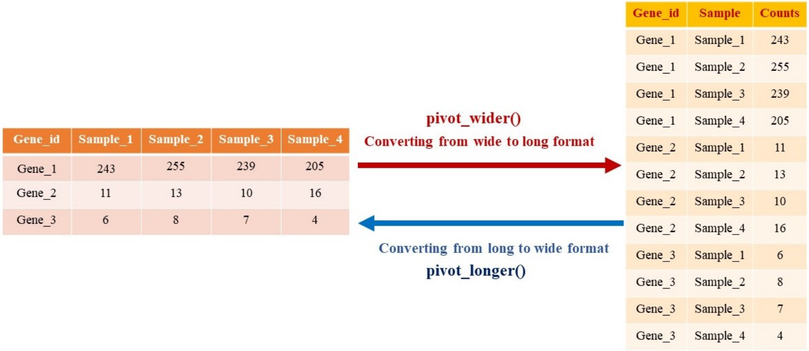
Counts table:



	gene_symbol	GSM1480291	GSM1480292	GSM1480293	GSM1480294
ENSMUSG00000000001	Gnai3	243.28596	255.66037	239.73819	217.10047
ENSMUSG00000000003	Pbsn	0	0	0	0
ENSMUSG00000000028	Cdc45	11.18453	13.78314	11.60091	4.2718
ENSMUSG00000000031	H19	6.30808	8.53042	7.09408	11.03901
ENSMUSG00000000037	Scml2	2.19217	4.66442	2.7959	2.49541
ENSMUSG00000000049	Apoh	0.22369	0.08404	0	0
ENSMUSG00000000056	Narf	11.27401	14.74964	26.16464	18.8213
ENSMUSG00000000058	Cav2	118.24288	112.70235	50.53489	63.40027
ENSMUSG00000000078	Klf6	2036.16657	2230.26276	1902.63241	1959.61407
ENSMUSG00000000085	Scmh1	33.68781	38.70204	9.18057	9.4318
ENSMUSG00000000088	Cox5a	126.92208	108.75231	141.96507	125.4894

Formatting the data

Converting from wide to long format:



Gene_id	Sample_1	Sample_2	Sample_3	Sample_4
Gene_1	243	255	239	205
Gene_2	11	13	10	16
Gene_3	6	8	7	4

pivot_wider()
Converting from wide to long format

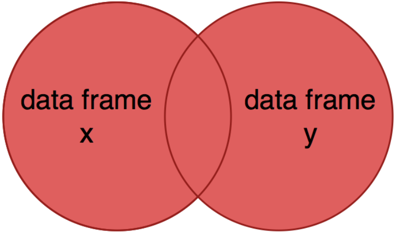
Converting from long to wide format
pivot_longer()

Gene_id	Sample	Counts
Gene_1	Sample_1	243
Gene_1	Sample_2	255
Gene_1	Sample_3	239
Gene_1	Sample_4	205
Gene_2	Sample_1	11
Gene_2	Sample_2	13
Gene_2	Sample_3	10
Gene_2	Sample_4	16
Gene_3	Sample_1	6
Gene_3	Sample_2	8
Gene_3	Sample_3	7
Gene_3	Sample_4	4

Formatting the data

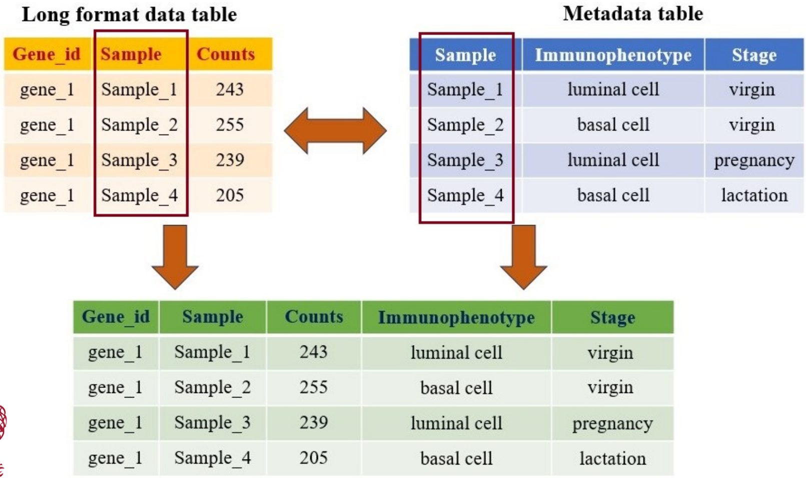
Joining (merging) datasets:

- The process involves combining datasets that share at least some of the same observations (rows) but have different variables (columns).
- Typically, there is one variable in common, called the **“key”** variable, shows which rows from one dataset match the rows of the other.



Formatting the data

Joining two tables:



Long format data table

Gene_id	Sample	Counts
gene_1	Sample_1	243
gene_1	Sample_2	255
gene_1	Sample_3	239
gene_1	Sample_4	205

Metadata table

Sample	Immunophenotype	Stage
Sample_1	luminal cell	virgin
Sample_2	basal cell	virgin
Sample_3	luminal cell	pregnancy
Sample_4	basal cell	lactation

Gene_id	Sample	Counts	Immunophenotype	Stage
gene_1	Sample_1	243	luminal cell	virgin
gene_1	Sample_2	255	basal cell	virgin
gene_1	Sample_3	239	luminal cell	pregnancy
gene_1	Sample_4	205	basal cell	lactation

Frame Title

Data handling and visualization in R

Data handling and visualization in R

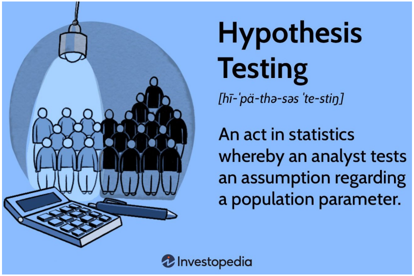
Link: https://colab.research.google.com/drive/1mRuWrMP87i8i9TQSS3VReyyGEtRZUehC?usp=sharing

Hypothesis Testing

Hypothesis Testing

Inferential statistics: is used to measure behavior in samples to learn more about the behavior in populations (often too large or inaccessible).

Hypothesis testing or **significance testing** is a method for testing a claim or hypothesis about a parameter in a population, using data measured in a sample.



Hypothesis Testing

[hī-ˈpä-thə-səs ˈte-stiŋ]

An act in statistics whereby an analyst tests an assumption regarding a population parameter.

Investopedia

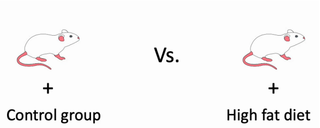
Hypothesis Testing

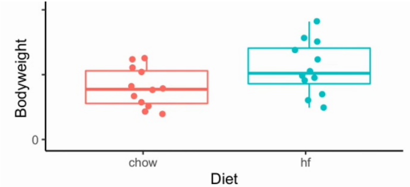
A hypothesis is

- an educated guess about something in the world around you.
- It should be testable, either by experiment or observation.
- For example a new treatment method we think might work for patients
  - Statement: If the patients receive counseling in addition to medication then their overall depression scale will decrease.
- A statistical hypothesis test is a method of statistical inference used to decide whether the data sufficiently supports a particular hypothesis.

Hypothesis Testing

Let's consider the mice weight example from Winzell and Ahrén [2004]. This study characterizes the high-fat diet–fed mouse as a model for impaired glucose tolerance (IGT) and type 2 diabetes.





Question: Is there a difference in weight between mice with control vs high-fat diet?

Steps of hypothesis testing

Ideally, we undertake the following steps:

- **Step 1:** State the hypotheses.
- **Step 2:** Set the criteria for a decision.
- **Step 3:** Compute the test statistic.
- **Step 4:** Make a decision.

State the hypotheses

Null Hypothesis (H0):

- is a statement about a population parameter, such as the population mean, that is assumed to be true.
- We will test whether the value stated in the null hypothesis is likely to be true.
- The only reason we are testing the null hypothesis is because we think it is wrong.

State the hypotheses

Alternative Hypothesis (H1):

- is a statement that directly contradicts a null hypothesis.
- states that the actual value of a population parameter is less than, greater than, or not equal to the value stated in the null hypothesis.

Null Hypothesis:

H_0; $\mu = 85\%$

Alternative Hypothesis:

H_1; $\mu \neq 85\%$

State the hypotheses

Mice example:

Null Hypothesis (H0):

there is no difference between the two diet groups.

Null model: $weight = mean\ (grand\ mean) + residuals$

Alternative Hypothesis (H1):

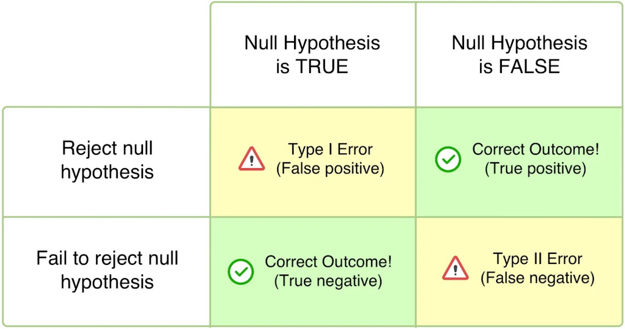
there is a difference between the two diet groups.

Alternative model: $weight = diet\ (group\ mean) + residuals$

A null hypothesis is **rejected** if the measured data is significantly unlikely to have occurred by chance.

Types of error

- Type I error, or α error, means rejecting the null hypothesis when the null hypothesis is true.
- Type II error, or β error, is the probability of retaining a null hypothesis that is actually false.

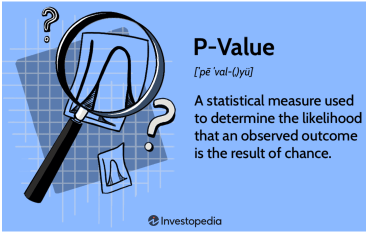


	Null Hypothesis is TRUE	Null Hypothesis is FALSE
Reject null hypothesis	⚠ Type I Error (False positive)	✓ Correct Outcome! (True positive)
Fail to reject null hypothesis	✓ Correct Outcome! (True negative)	⚠ Type II Error (False negative)

The criteria for a decision: P-value

A p-value (or probability value)::

- is the probability of an α error.
- is a number describing how likely it is that your data would have occurred by random chance.
- The lower the p-value, the greater the statistical significance of the observed difference.
- a p-value doesn't tell us if the null hypothesis is true or false. It's a piece of evidence, not a definitive proof.



P-Value

['pē 'val-(ˌ)yü]

A statistical measure used to determine the likelihood that an observed outcome is the result of chance.

The criteria for a decision: statistical significance

Statistical significance: or an α level is the level of significance or criterion for a hypothesis test.

- It is the largest probability of committing a Type I error (the highest value of p) that we will allow and still decide to reject the null hypothesis.
- In hypothesis testing, if $p \leq \alpha$, reject the null hypothesis. If $p > \alpha$, fail to reject (retain) the null hypothesis.

We directly control for the probability of a Type I error by stating an α level.

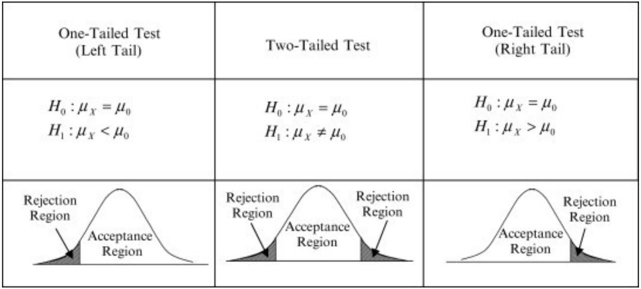
Example: Binomial test

Scenario:

- The known prevalence rate for a disease is $r = 4\%$.
- Sample: 100 test persons, $X = 9$ of them have the disease.
- H0: The prevalence in the test group is also $r = 4\%$.
- H1: The prevalence in the test group differs from $r = 4\%$.
- significance level is $\alpha = 0.05$.
- if $r = 4\%$, the probability of observing 9 or more persons with disease is $P(X \geq 9) = 0.016$, rather unlikely.

Decision: $p \leq \alpha$, we can reject the null hypothesis .

Hypothesis Types

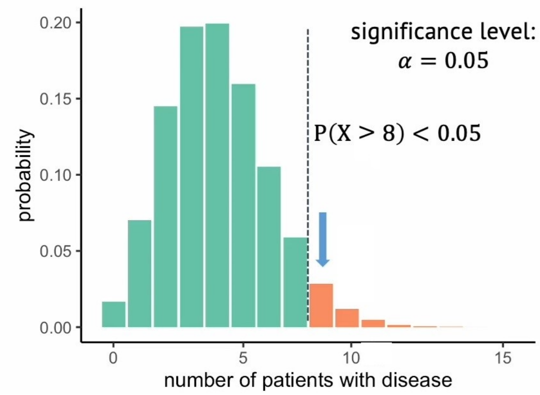


One-Tailed Test (Left Tail)	Two-Tailed Test	One-Tailed Test (Right Tail)
$H_0 : \mu_X = \mu_0$ $H_1 : \mu_X < \mu_0$	$H_0 : \mu_X = \mu_0$ $H_1 : \mu_X \neq \mu_0$	$H_0 : \mu_X = \mu_0$ $H_1 : \mu_X > \mu_0$
Rejection Region Acceptance Region	Rejection Region Acceptance Region Rejection Region	Acceptance Region Rejection Region

Hypothesis Types: Example

One-sided Binomial test, look only in one direction:

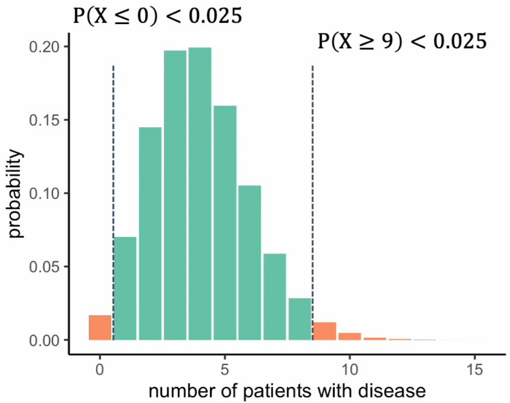
$$H1 : r > 0.04 \ \textbf{or} \ H1 : r < 0.04$$



Hypothesis Types: Example

Two-sided Binomial test, look in both directions. Which number of test persons are very unlikely/extreme, assuming H0 is true? $H1 : r \neq 0.04$

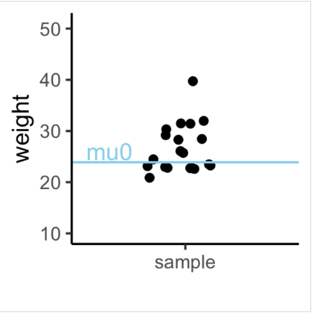
$$P(X = 0) = 0.017, \;\; P(X > 8) = 0.019$$

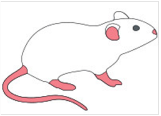


One-sample t-test

It is used for comparing one group's average to a known average.

Example: We now want to know whether the mice weights in the test group that have been fed a high-fat diet (black dots) differ significantly from a known average mouse weight (blue line).

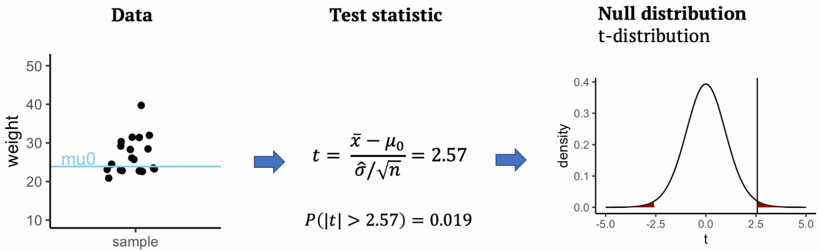




One-sample t-test

t-statistics for one-sample: $t = \frac{\bar{X} - \mu_0}{\frac{\hat{\sigma}}{\sqrt{n}}}$.

The larger the t-statistic, the more likely that your results will be statistically significant



Data

Test statistic

$$t = \frac{\bar{x} - \mu_0}{\hat{\sigma}/\sqrt{n}} = 2.57$$

$$P(|t| > 2.57) = 0.019$$

Null distribution
t-distribution

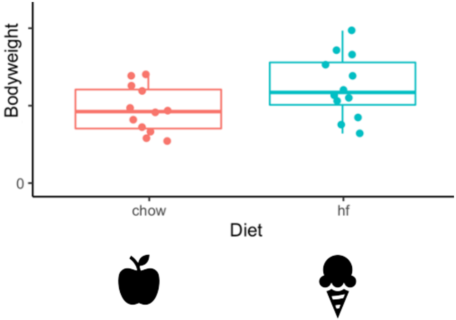
Conclusion: We can reject H0 at a 5% significance level, i.e. the data mean is different from μ_0.

Two-sample t-test

It is used for comparing two groups with equal variances and sample sizes.

Example: a high-fat diet (Sample A) vs a control diet (Sample B).

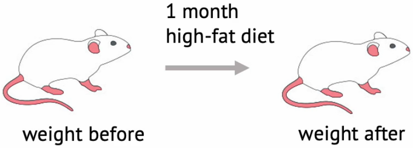
t-statistics for two samples: $t = \frac{\bar{X}_A - \bar{X}_B}{SE}$ with $SE = \sqrt{\frac{\hat{\sigma}_A^2 + \hat{\sigma}_B^2}{n}}$

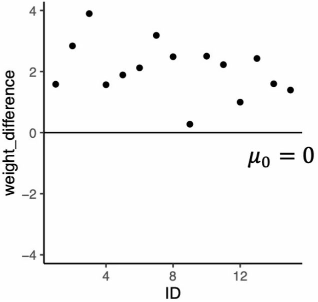


Pairing data t-test

Example: The weight of some mice is measured before and after 1 month of a high-fat diet. The issue with doing the two-sample t-test is the high variance between individual mice weights.

One-sample t-test: (H0) the mean weight gain/loss is equal to zero





Hypothesis testing in R

Link: https://colab.research.google.com/drive/1mRuWrMP87i8i9TQSS3VReyyGEtRZUehC?usp=sharing

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