



Outline/Intended Learning Outcome (ILOs)

Day 2: Hypothesis testing, tests for continuous responses, multiple testing

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Hypothesis testing

ILO: to describe the principles and logic of hypothesis testing

One and two sample tests for continuous responses: t-test

ILO: to identify when, how and why to use a t-test

ILO: to define a p-value and contrast its use with that of a confidence interval

Power and Sample size calculation

ILO: to identify why and how to make power and sample size calculations

ILO: to analyse their strengths and limitations

Multiple testing

ILO: to describe the multiple testing problem and employ basic remedies

Nonparametric test: Wilcoxon

ILO: to contrast pros and cons of Wilcoxon vs t-test



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Case: cow milk data



- ▶ **Research question:**
Should cows be fed with **Barley** or **Lupin**, to produce the best milk?
- ▶ **Outcome:**
protein level of the milk (%) at 12 weeks after calving.

Statistical aim: provide a yes/no answer about the **population supported by the observed data (sample)** while controlling the risks of a "false finding", via a **Hypothesis test**.¹

Research question and Null hypothesis

- ▶ A hypothesis test aims to answer a very **precise & specific** research question.

Case: Is there a **difference in (population) mean** level of protein between cows fed with lupin and barley, at 12 weeks?

- ▶ The **null hypothesis** \mathcal{H}_0 of the test should reflect it and state the **opposite** of what you aim to prove.

- ▶ **Scientific hypothesis:** there is a difference.
- ▶ **Null hypothesis:** there is **no** difference.

Choosing the opposite is important to appropriately control the **risk of wrong conclusion**.

¹Note: important complementary information is given by the confidence interval of the effect size. It will be emphasized shortly.



Hypothesis testing and risks of false conclusions



Case:

- ▶ **Type-I error:** conclude a difference although it does not exist.
→ **False positive finding**
- ▶ **Type-II error:** do not conclude to a difference although it exists.
→ **False negative finding**

The logic of hypothesis testing

1. **Assume** that the data have been generated in a world in which the **null hypothesis is true**.
2. Under this assumption, **calculate how unlikely it is to obtain some results that contradict the null hypothesis** as least as much as those obtained with your data (i.e., compute the p-value).
3. Reject the null hypothesis if this is unlikely 'enough'.

- ▶ Similar to a proof by contradiction.
- ▶ Computation in step 2. depends on the type of observed data.



Hypothesis testing and risk control

We want to ensure that the risk of wrongly rejecting the null hypothesis (α) is small (often 5%), i.e. **a small risk of a false scientific finding**.

Reasoning: the data need to be convincing enough to support the (new) research finding.

Limitation: it might be difficult to have enough data to support a (new) finding (→ power).



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Case: cow milk data

Data from $n = 25$ (Barley)+27 (Lupin) cows:

protein	Diet	
3.28	lupins	
3.04	barley	
3.07	barley	
2.92	barley	
3.29	lupins	
3.18	lupins	
Mean (SD):		Barley Lupin
3.43 (0.31)	3.21 (0.27)	

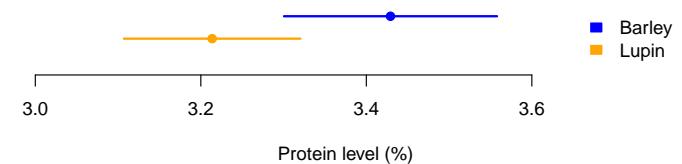
etc...

- ▶ Is the **difference** observed in the data **sample** large enough to conclude to a difference in the **population**?

First approach: not optimal for testing for a difference

Comparison of 95% confidence intervals:

- ▶ Lupin: [3.11;3.32]
- ▶ Barley: [3.30;3.56]



We cannot conclude on the significance of the difference

(see slides lecture 1).

But the two CI can be interesting to report anyway.

A better approach

Compute:

- ▶ p-value for the difference in mean.
- ▶ confidence interval for the difference in mean.

Two-sample t-test (1/2)

Model assumptions: (1 & 2 are important, 3 not always)

1. The two samples are **independent** (no pairing).
2. Observations from each sample are **independent**.
3. Observations are normally distributed.

To test the null hypothesis $\mathcal{H}_0 : \mu_1 = \mu_2$, i.e. the population means are the same in the two populations, we compute the **t-statistic**.

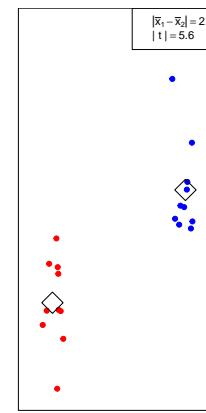
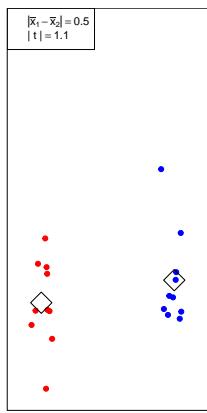
$$t = \frac{\bar{x}_1 - \bar{x}_2}{s.e.(\bar{x}_1 - \bar{x}_2)}$$

where the standard error is $s.e.(\bar{x}_1 - \bar{x}_2) = \sqrt{s_1^2/n_1 + s_2^2/n_2}$.

The value t quantifies how large the (sample) difference $(\bar{x}_1 - \bar{x}_2)$ is **relative** to the amount of **information** provided by the data $(s.e.(\bar{x}_1 - \bar{x}_2))$ and is used to compute a p-value.

Appendix: t-statistic intuition (1/3)

- ▶ For given standard deviations s_1 and s_2 and sample sizes n_1 and n_2 , **the larger the difference in mean $\bar{x}_1 - \bar{x}_2$ and the larger the t-statistic (absolute values).**



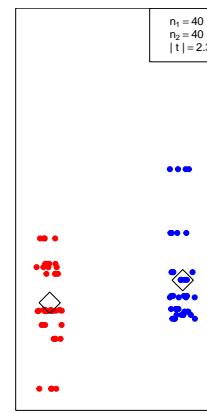
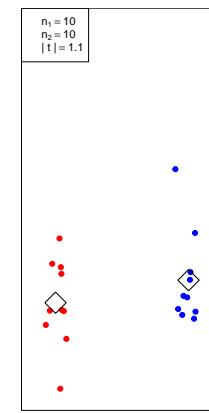
- ▶ **It makes sense:** the right plot is more convincing than there is a difference in mean than the left plot.

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Appendix: t-statistic intuition (2/3)

- ▶ For given standard deviations s_1 and s_2 and difference in mean $\bar{x}_1 - \bar{x}_2$, **the larger the sample sizes n_1 and n_2 and the larger the t-statistic (absolute values).**



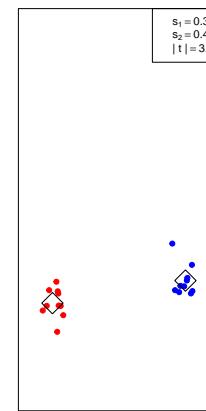
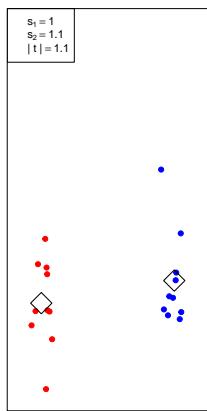
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Appendix: t-statistic intuition (3/3)

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- ▶ **It makes sense:** the right plot is more convincing than there is a difference in mean than the left plot.

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Two-sample t-test (2/2)

The key idea to use the t -statistics is that under the model assumption, it follows a specific distribution² whatever the value of the (population) means (μ_1, μ_2) and standard deviations (σ_1, σ_2) in each group.

Hence we can assume $\mu_1 = \mu_2$ and calculate how **unlikely** it is to obtain a t value that **contradicts the null hypothesis as least as much** as that obtained with your data, that is, we can compute a **p-value**.

The larger $|t|$ the more the data contradict $\mathcal{H}_0 : \mu_1 = \mu_2$.

p-value = $P(|T| > |t|)$, where T is a random variable that follows the t-distribution.

²the t-distribution, aka Student's distribution, which depends on the two sample sizes n_1 and n_2 , already encountered in Lecture 1.



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The p-value (1/2)

Informal definition:

The p-value is “*the probability of seeing a result as extreme as your observed result, when the null model is true.*”³

- ▶ “*result*”= test statistic, i.e., a single value that (“cleverly”) summarizes the data.
- ▶ “*extreme*”= unlikely/unexpected.

Interpretation:

1. If the **p-value is small** the data are at odds with the null hypothesis⁴ and the finding is said to be statistically **significant**.
2. If the **p-value is large**, the finding is said to be not statistically **significant**.

³Westfall & Henning. Understanding advanced statistical methods. CRC Press, 2013 (page 405).
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⁴i.e. either the null hypothesis is not true or a very rare event has occurred.

The p-value (2/2)

Interpretation:

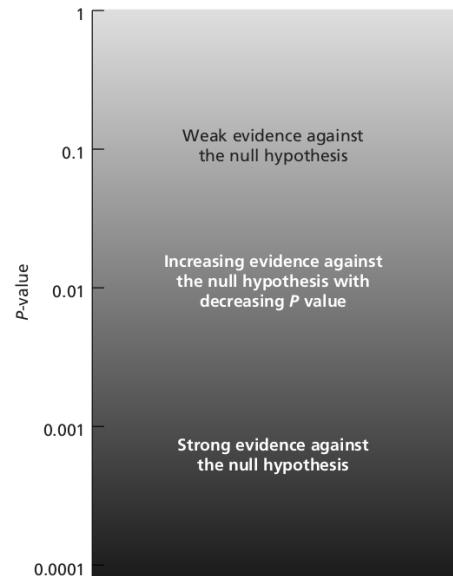
3. We imagine a large number of **repetitions** of the study with the null hypothesis being true and define the **p-value** as the **proportion** of these studies which provide **less support** for the **null hypothesis** than the **data actually observed**.

Recommendations:

- ▶ Traditionally the value $p=5\%$ has been used to divide “**significant**” from “**non-significant**” results, but **good practice is to report the actual p-value**.
- ▶ The choice of the threshold to claim significance (often $p=5\%$) should be **prespecified**.

p-value and strength of evidence

The smaller the p-value, the stronger the evidence against the null hypothesis.



Case: Two-sample t-test

- ▶ $\bar{x}_1 = 3.43, \bar{x}_2 = 3.21$
- ▶ $\bar{x}_1 - \bar{x}_2 = 0.22$
- ▶ $n_1 = 25, n_2 = 27$
- ▶ $s_1 = 0.31, s_2 = 0.27$
- ▶ $s.e.(\bar{x}_1 - \bar{x}_2) = 0.081$
- ▶ $t = 2.66$
- ▶ $p\text{-value} = P(|T| > |t|) = 0.011$

We conclude that there is a **significant difference** in mean protein level of the milk between cows fed with barley and lupin ($p=0.011$).

Don't misinterpret p-values!

Widespread misunderstandings called for clarifications:

- The p-value is **not** the probability of \mathcal{H}_0 being true.

"Researchers often wish to turn a p-value into a statement about the truth of a null hypothesis, or about the probability that random chance produced the observed data. The p-value is neither."⁵

This is because $P(\text{extreme result}|\mathcal{H}_0) \neq P(\mathcal{H}_0|\text{extreme result})$, which relates to the concept of "invalid inversions".⁶

⁵ Wasserstein & Lazar. "The ASA statement on p-values: context, process, and purpose." Am. Stat. 70.2 (2016): 129-133.

⁶ Simiary, "The probability that a woman has breast cancer is small. The probability that someone with breast cancer is a woman is large. Obvious- but some invalid inversions are not so easily seen." Senn, S.J. (2013). Invalid inversion. Significance 10 (2): 40-42.

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- Observing a p-value $\leq 5\%$ does not mean that the positive finding is easy to reproduce!⁷

As an example, suppose you conduct a study and find a significant p-value of $p = 5\%$. You try to replicate your positive finding by conducting the exact same study again. What is your chance to get a significant p-value ($\leq 5\%$) again? To calculate that, you need to know the true treatment effect " $\mu_1 - \mu_2$ ", which you do not know, of course. But what about if we assume that it is what you have estimated in your (first) study, i.e. $\bar{x}_1 - \bar{x}_0$?

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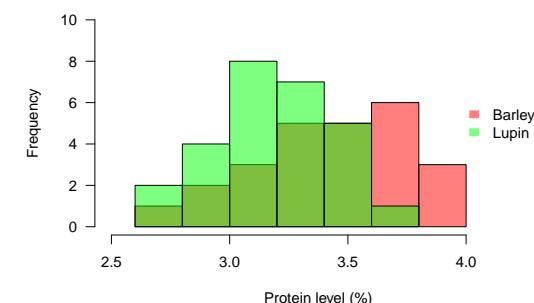
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Normality assumption

Normality should be checked for **each sample separately** (using histograms or qqplots).



But, when sample sizes n_1 and n_2 are both large enough (say > 15) normality is **not important**⁸.

However, **skewed data can be transformed** to facilitate the interpretation and reduce the influence of outliers (this should be pre-specified).

⁸ due to the central limit theorem.

Confidence interval of the difference

Good practice: report an estimate of the mean difference and a **95% confidence interval**.

$$\bar{x}_1 - \bar{x}_2 \pm t_{df} \cdot s.e.(\bar{x}_1 - \bar{x}_2)$$

- ▶ df : degree of freedom $\approx n_1 + n_2 - 2$ when $n_1 = n_2$ and $s_1 = s_2$.
- ▶ $t_{df} \approx 1.96$ when n_1 and n_2 are large (say ≥ 15).
- ▶ software will take care.

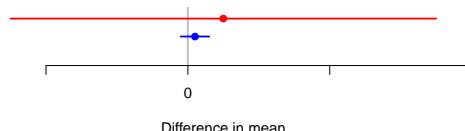
Case: mean difference of -0.22 (CI-95% = [0.05;0.38]; p-value = 0.011).

Confidence interval vs p-value

- ▶ if 0 is $\left\{ \begin{array}{l} \text{in} \\ \text{not in} \end{array} \right\}$ the CI, then the difference $\left\{ \begin{array}{l} \text{is not} \\ \text{is} \end{array} \right\}$ significant.
- ▶ We can tell if the test is significant from looking at the CI, but we can't guess the CI from knowing the p-value.

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- ▶ We can tell if the test is significant from looking at the CI, but we can't guess the CI from knowing the p-value.
- ▶ A **wide** 95% that includes 0 suggests "**lack/absence of evidence**".
- ▶ A **narrow** 95% that includes 0 suggests "**evidence of absence**" of difference (or existence of a "tiny one", if any).



"In particular, large p values should not be interpreted as showing no association or no effect: absence of evidence is not evidence of absence. Only a very narrow interval estimate near the null value (0 for differences, 1 for ratios) warrants inferring that the study found no important association or effect."

A common incorrect interpretation!

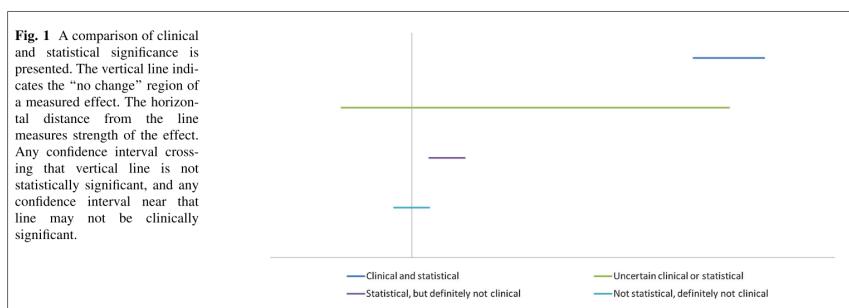
"Based on the experience of reviewing statistics in more than **1000 manuscripts submitted to The Lancet Group** of journals over the past 3 years, this Correspondence provides guidance to **commonly encountered statistical deficiencies** in reports and how to avoid them (panel)." ⁹

Panel: Basic recommendations for accurate reporting of statistics

- Do not report results as showing no effect, unless all effects inside the interval estimate are clinically unimportant.



Clinical relevance versus statistical significance



Clinical relevance \neq statistical significance !

Source: Maltenfort & Díaz-Ledezma. Statistics In Brief: Minimum Clinically Important Difference—Availability of Reliable Estimates. Clinical Orthopaedics and Related Research 475(4):p 933-946, 2017.

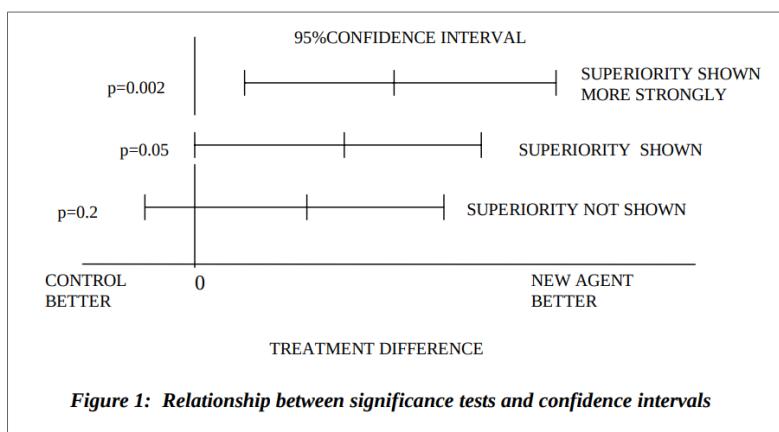
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Appendix: Need for CIs (2/2)



Appendix: Need for CIs (1/2)

p-Values are only one tool for assessing evidence. When reporting the results of a clinical trial, CIs should always be reported to identify effect sizes that can be "ruled out" (i.e., effect sizes that are inconsistent with the data). If a p-value is significant, implying an effect, then the next natural question is "what is the effect?" CIs directly address this question. If a p-value is not significant, implying that you were not able to rule out the possibility of "no effect," then the next natural question is "what effects could be ruled out?"

CIs again directly address this question. The under-reporting of CIs is a serious flaw in the medical literature.

CIs are not a replacement for p-values but instead should be provided with p-values. p-values are still very useful tools particularly when assessing trends and interactions.

"Fundamental Concepts for New Clinical Trialists", by Evans and Ting (2016), pages 216–217, Section 8.3.4.2 "Need for CIs".

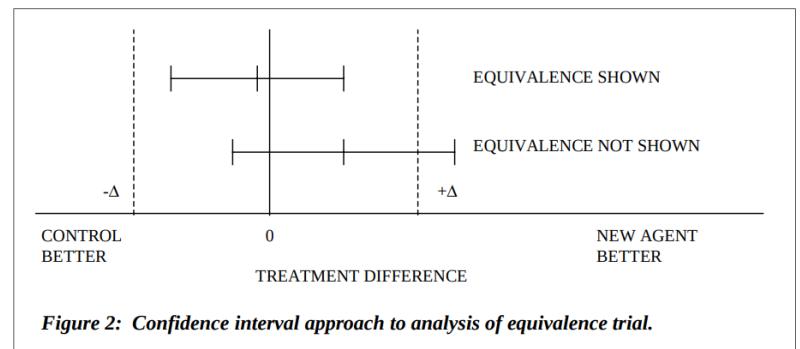
"Avoid relying solely on statistical hypothesis testing, such as P values, which fail to convey important information about effect size and precision of estimates" in "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals", Updated January 2025, see <https://www.icmje.org>.

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Appendix: CIs for evidence of "clinical equivalence"

E.g.: $\Delta = 5 \text{ mmHg}$ in blood pressure



EMA scientific guidelines "Points to consider on switching between superiority and non-inferiority" (2000), https://www.ema.europa.eu/en/documents/scientific-guideline/points-consider-switching-between-superiority-and-non-inferiority_en.pdf

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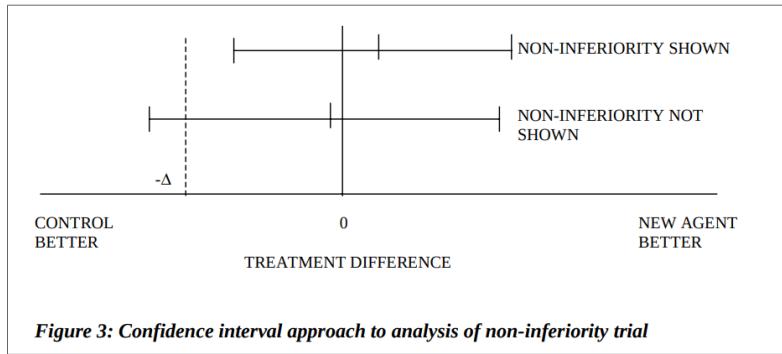
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Appendix: CIs for evidence of “clinical non-inferiority”

E.g.: $\Delta = 5 \text{ mmHg}$ in blood pressure



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https://www.ema.europa.eu/en/documents/scientific-guideline/points-consider-switching-between-superiority-and-non-inferiority_en.pdf

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One-sample example

Research question:

Is the mean protein level of the milk similar at 1 and 12 weeks after calving, for cows fed with Barley?

Data ($t_1 - t_{12}$):

Cow	Diff
B01	-0.08
B02	-0.03
B03	1.06
B04	0.48
B05	0.49
B06	0.74

etc...

Null hypothesis:

The mean difference between protein level at 1 and 12 weeks is zero ($H_0 : \mu = 0$).

One-sample test because only one group of ($n=25$) cows (barley).

Two versions of the two-sample t-test

“Classical” Student’s t-test (not recommended):

- ▶ Original t-test, described in many basic textbooks.
- ▶ Additional assumption¹⁰ of equal standard deviations $\sigma_1 = \sigma_2$.
- ▶ Different formula for s.e. and degrees of freedom ($df = n_1 + n_2 - 2$).

Welch’s t-test (the presented one, recommended):

- ▶ No assumption of equal standard deviations: less restrictive.
- ▶ Formula for degrees of freedom more complicated, but software take care.
- ▶ Default in R.

¹⁰ Note: with equal group sizes, i.e. when $n_1 = n_2$, which is typically the case in experimental research, then this assumption is not important at all. See e.g. Julious SA. Why do we use pooled variance analysis of variance? *Pharmaceutical Statistics*. 2005 Jan;4(1):5-8.

One-sample t-test

The **t-test statistic** measures the distance between the sample mean and the assumed population mean μ under H_0 in units of the standard error:

$$t = \frac{\bar{x} - \mu}{s/\sqrt{n}}$$

If $|t|$ is large, the data “contradict” the null hypothesis.

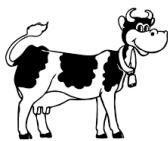
$$\text{p-value} = P(|T| > |t|)$$

where T is a random variable that follows the t-distribution with $n - 1$ degrees of freedom.

- ▶ similar to the computation of the confidence intervals for the mean.
- ▶ $\text{p-value} \leq 5\% \iff \mu \text{ not in } 95\% \text{ CI.}$

One-sample t-test: example results

- $\bar{x} = 0.46$
- $n = 25$
- $s = 0.31$
- $t = 7.43$
- $p\text{-value} = P(|T| > |t|) < 0.001$ (for $H_0 : \mu = 0$)



We conclude that there is a **significant difference** in mean protein level of the milk at 1 and 12 weeks after calving, for cows fed with barley ($p < 0.001$).

Reminder (see Lecture 1):

we compute the 95% CI as $\bar{x} \pm t_{n-1} \cdot s / \sqrt{n}$, which here leads to [0.33; 0.58] (and does not include 0).

Note: this one-sample t-test corresponds to a **paired t-test**¹¹.

¹¹ two samples of observations (two times) paired by cow. More on Lecture 8.

Power

The **power** of a test is the **chance** of obtaining a **significant result** when the null hypothesis is indeed false.

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Nonparametric test: Wilcoxon

ILO: to contrast pros and cons of Wilcoxon vs t-test

Power

Power

The **power** of a test is the **chance** of obtaining a **significant result** when the null hypothesis is indeed false.

- Power = $1 - \beta$, i.e. 1 minus the risk of a “false negative” result (β), i.e. 1 minus risk of Type-II error.
- Although we can control the type-I error ($\alpha = 5\%$) by appropriately computing the p-value and comparing it to 5%, the computation does not control the risk of type-II error, β .

- The power of a two-sample t-test depends on:

- **sample sizes** n_1 and n_2 (the larger the better).
- **standard deviations** σ_1 and σ_2 (i.e. variability, the smaller the better).
- **difference in mean** $\delta = |\mu_1 - \mu_2|$ (i.e. effect size, the larger the better).

Textbook power formula (approximation for two-sample t-test)

$$\delta = (z_{1-\beta} - z_{\alpha/2}) \sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}$$

- ▶ $z_{\alpha/2} = -1.96$ for $\alpha = 5\%$.¹²
- ▶ $z_{1-\beta} = 0.84$ and 1.28 for $1 - \beta = 80\%$ and 90% .
- ▶ maximal power when $n_1 = n_2$, for a given total sample size $n_1 + n_2$, when $\sigma_1 = \sigma_2$.

Useful for computing:

- ▶ **Sample size:** $n_1 = n_2$ for given “guesses” of σ_1 , σ_2 and δ and desired $1 - \beta$ and α .
- ▶ **Power for a given budget/sample size:** $1 - \beta$ for “guesses” of σ_1 , σ_2 and δ and desired n_1 , n_2 and α .
- ▶ **Least detectable difference:** δ for given n_1 and n_2 , “guesses” of σ_1 and σ_2 and desired α and minimal power $1 - \beta$.

^{37 / 64} ¹² where z_γ is the γ -quantile of a standard normal distribution.

Sample size calculation: which difference δ to use?

Principled choices:

- ▶ expected/hypothesized difference.
- ▶ minimum (clinically) relevant difference.

But **small difference are difficult to detect** and may require a large sample size, with consequences on the budget, study length, etc.

Pragmatic choice: smallest difference “disappointing” to overlook.

If this still indicates a too large sample size, then discuss with your supervisor (try to avoid wasting time/money).

Use a software ! (e.g. R)

Often it is “good enough” to assume $\sigma_1 = \sigma_2$ and then sensible to choose $n_1 = n_2$. Then standard software can be used, e.g. with R¹³:

```
power.t.test(power = .80, delta = 0.5)
```

Two-sample t test power calculation

```
n = 63.76576
delta = 0.5
sd = 1
sig.level = 0.05
power = 0.8
alternative = two.sided
```

NOTE: n is number in *each* group

- ▶ $n_1 = n_2 = 64$ subjects needed to detect 1/2 sd difference¹⁴.

^{38 / 64} ¹³ slightly more precise calculation performed than using the textbook formula.

^{38 / 64} ¹⁴ Note: It holds for whatever $\sigma_1 = \sigma_2$ and δ , as long as δ/σ_1 , the “signal-to-noise ratio”, is 1/2.

Which guesses for the standard deviations?

For calculations, we need a “guess” of outcome variability, i.e. σ_1 , σ_2 .

- ▶ Estimate from previous studies from your research group or published in the literature, but beware of statistical uncertainty!
- ▶ Expert guess (supervisor/senior collaborators); asking them about the expected normal range¹⁵ (and divide its width by 4) can help can be better to guess σ than directly asking about σ .

Recommended practice:

- ▶ use several likely values to do several calculations.
- ▶ see how changes affect the results and discuss with your collaborators.
- ▶ be conservative (when appropriate).
- ▶ consider ethical issues (whenever relevant).

^{39 / 64} ¹⁵ Eg., ask “In which interval do you expect that approximately 95% of the outcome values will fall?” Personal experience suggests that this provides substantially more accurate guesses than asking the expected value of σ directly. Rationale is that the interval “mean $\pm 2\sigma$ ” includes 95% of the observations, if the data are normally distributed, and more than 75% in any case

Least detectable difference: sensitivity to σ

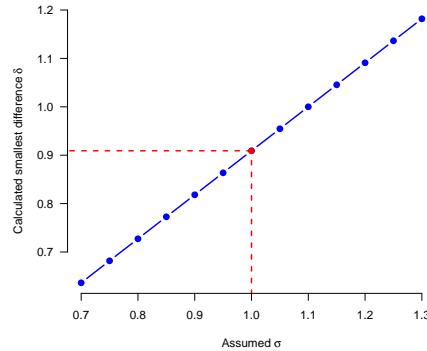
Example: my grant (money/time) can finance a sample size of $n = 40$ (i.e. 20 per group). **What is the smallest difference I can hope to show different from zero, with a decent power (e.g. 80%)?**

```
power.t.test(n=20,sd=1,power=0.80)
```

Two-sample t test power calculation

```
n = 20
delta = 0.9091306
sd = 1
sig.level = 0.05
power = 0.8
alternative = two.sided
```

NOTE: n is number in *each* group



Note: textbook formula gives $\delta = 2.8 \cdot \sigma \cdot \sqrt{2/20}$.

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ILO: to contrast pros and cons of Wilcoxon vs t-test

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Power: sensitivity to σ

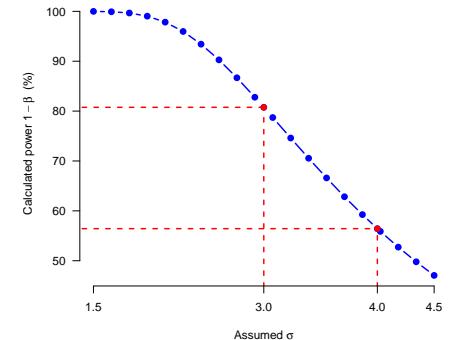
Example: an initial calculation suggests $n = 74$ (i.e. 37 per group), for the Minimal Clinically Important Difference (MCID) $\delta = 2$, with our best expert guess $\sigma = 3$ (with 80% power). **But what does the power become if we over or underestimate σ by up to 50%?**

```
power.t.test(sd=4,delta=2,n=37)
```

Two-sample t test power calculation

```
n = 37
delta = 2
sd = 4
sig.level = 0.05
power = 0.5642987
alternative = two.sided
```

NOTE: n is number in *each* group



Note: textbook formula gives $z_{1-\beta} = (2/\sigma) \cdot (\sqrt{37}/\sqrt{2}) - 1.96$ and tables and software give $z_{1-\beta} = 1.64, 1.28, 0.84, 0.25, -0.52$ for $1 - \beta = 95, 90, 80, 60$ and 30%, respectively.

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A multiple testing example



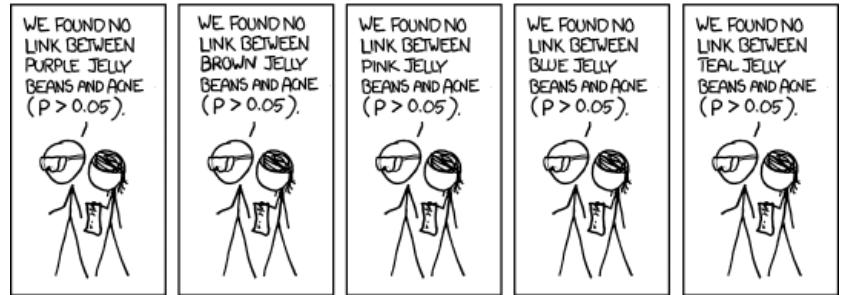
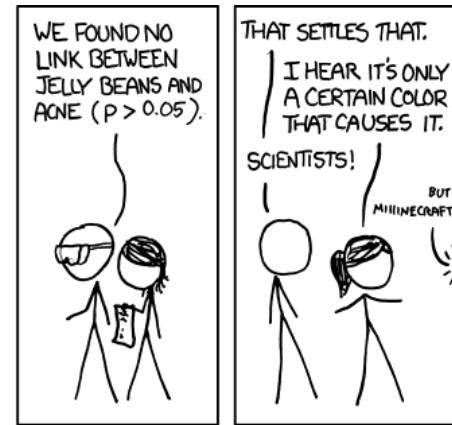
Are jelly beans associated with acne?



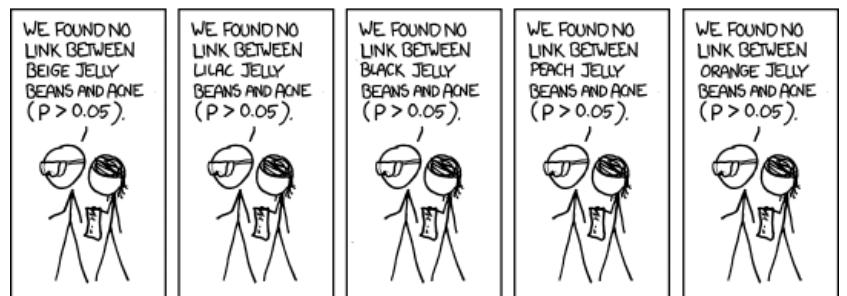
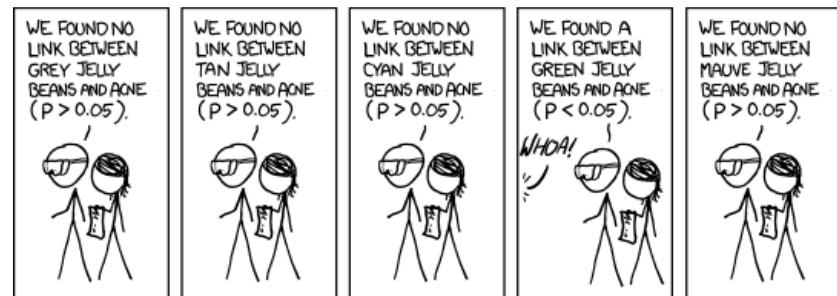
(cartoon from: <https://xkcd.com/882/>)

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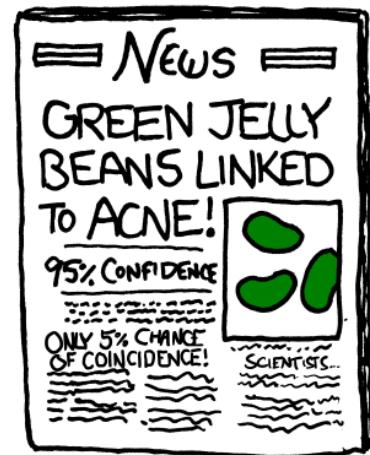
- ▶ First test is not significant.
- ▶ Move on to other tests.



- ▶ Four more tests are not significant, but one is significant (Green!).
- ▶ Move on to other tests.

- ▶ Five more tests are not significant.
- ▶ Stop testing.





Multiple testing issue

- ▶ Conclude.
- ▶ The risk of type-I error of **each** test is controlled (usually at 5%).
- ▶ i.e. thinking of each hypothesis test separately, each corresponding to a specific research question and specific study, the risk of false positive finding is controlled for each of them.
- ▶ But, if we consider them part of the same study and consider that we have a finding if at least one test is significant, then we do not control the risk of false positive finding.
- ▶ i.e. **the risk of having at least one significant p-value although there is no association is not controlled.**

Is the conclusion correct? Why?

FWER in the example

We have computed $K = 16$ different p-values. For simplicity, we assume that the data to compute each of them are different (independent).

$$\begin{aligned}
 \text{FWER} &= P(\text{at least one of the } K \text{ p-values are significant}) \\
 &= 1 - P(\text{none of the } K \text{ p-values are significant}) \\
 &= 1 - P(\text{1st is not significant}) \times \cdots \times P(K\text{-th is not significant}) \\
 &= 1 - (1 - 0.05) \times \cdots \times (1 - 0.05) \quad (\text{as no association exists}) \\
 &= 1 - (1 - 0.05)^K
 \end{aligned}$$

K	1	2	3	4	5	10	16	20	50
FWER (%)	5	10	14	18	23	40	56	64	92

Cartoon: 56% chance of at least one significant false finding if no association exists.

Family-wise error rate (FWER): probability of making one or more false discoveries when performing multiple hypotheses tests



FWER control

When we plan to compute $K \geq 1$ p-values, we can adjust their computation to control the FWER.

Bonferroni adjustment:

- ▶ adjusted p-value = $K \times$ original p-value
- ▶ adjusted significance level = α/K .¹⁶



¹⁶ Can be used to compute adjusted confidence intervals.

Not allowed to keep testing until one significant result pops up and then multiply all p-values by the number of tests performed.



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Intuition:

- ▶ equally share/split the original significance level α between the tests.
- ▶ the “total” risk of error (FWER) cannot exceed the sum of the errors of each test.

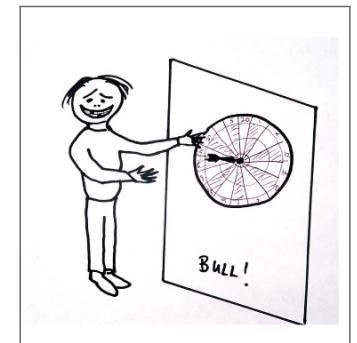
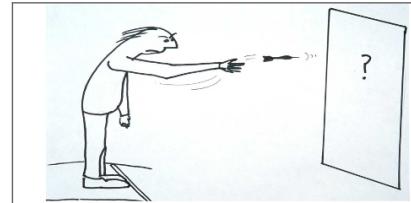
Remarks:

- ▶ always works: no specific assumption.
- ▶ but only works if we prespecify the analysis with K tests.¹⁷

¹⁶ Can be used to compute adjusted confidence intervals.

¹⁷ Not allowed to keep testing until one significant result pops up and then multiply all p-values by the number of tests performed.

Prespecification matters



- ▶ Concluding significance without prespecification is like drawing a dart-board around where the dart lands.
- ▶ “If you torture the data long enough, it will confess to anything.”¹⁸

Bonferroni-Holm adjusted p-values

1. sort the p-values: $p_{(1)} \leq p_{(2)} \leq \dots \leq p_{(K)}$
2. adjust the first as with Bonferroni, i.e. $\tilde{p}_{(1)} = K \cdot p_{(1)}$ and others as

$$\tilde{p}_{(i)} = \max \left\{ \tilde{p}_{(i-1)}, (K - i + 1) \cdot p_{(i)} \right\}$$

(≈ multiply the 1st by K , the 2nd by $K - 1$, the 3rd by $K - 2$,)

Remarks:

- ▶ same as for Bonferroni (no specific assumption).
- ▶ we cannot compute corresponding adjusted significance levels and adjusted confidence intervals.
- ▶ less conservative than Bonferroni, i.e. adjusted p-values are always smaller.

Example

We compare 6 doses of treatments (10-60 mg) to placebo (0 mg).

Comparison	60 mg	50 mg	40mg	30mg	20mg	10mg
Original p-value	0.005	0.009	0.1	0.15	0.3	0.6
Bonferroni	0.03	0.054	0.6	0.9	1	1
Bonferroni-Holm	0.03	0.045	0.4	0.45	0.6	0.6

Note: we “truncate” the p-value to 1.

FWER vs FDR (1/2)

Controlling the **FWER** is important in “**confirmatory**” studies.

- When there is a clear **prespecified** scientific hypothesis and the aim is to “prove” it. E.g. **clinical trial**.

Controlling the **FDR** is often better suited in “**exploratory**” studies.

- When nice data are available, but **no specific research questions** / scientific hypotheses. You want to look at many associations and report findings which are “likely enough” true findings. E.g. **Genomics**.

False discovery rate (FDR): expected proportion of falsely rejected hypotheses among the rejected hypotheses.

Digression: confirmatory versus exploratory research

Exploratory research	Confirmatory research
No hypothesis required/hypothesis can be vague Generate new hypothesis from data High sensitivity desired, i.e. minimising the risk of false negatives Suitable for making new discoveries and finding the unexpected For example: Testing of new compounds in mice	Clear hypothesis required Test <i>a priori</i> hypothesis with new data High specificity desired, i.e. minimising the risk of false positives Suitable for establishing strong evidence and confirming the expected For example: Assessing the efficacy of a drug in humans

“It is essential to distinguish between exploratory and confirmatory research, and they are equally important to the scientific enterprise. Finding the questions to ask is at least as crucial as answering them, if not more so. But when the two concepts get confused, and the two worlds collide, the fallout can be disastrous”¹⁹

FWER vs FDR (2/2)

Hypotheses	Not rejected	Rejected	Total
True	U	V	K ₀
False	T	S	K - K ₀
Total	W	R	K

- $FWER = P(V > 0)$
- $FDR = E(V/R)$ (where here we set $V/R = 0$ if $R = 0$).
- controlling the **FDR** is less conservative than controlling the **FWER**: p-values adjusted to control the FDR are smaller than those adjusted to control the FWER.
- See **Benjamini-Hochberg** (1995) method to control FDR at e.g. 5%.

Multiple testing corrections: none can be fine/best !

It is not always needed, or even possible, to adjust for multiple testing.

“In exploratory studies without prespecified hypotheses there is typically no clear structure in the multiple tests, so an appropriate multiple test adjustment is difficult or even impossible. Hence, we prefer that data of exploratory studies are analysed without multiplicity adjustment. However, ‘significant’ results based on exploratory analyses should be clearly labelled as exploratory results. To confirm these results, the corresponding hypotheses have to be tested in confirmatory studies.”

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Case: gene expression

► Research question:

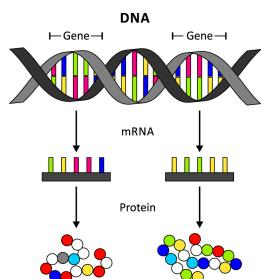
Is the length of the candidate gene NACP associated with the level of expressed alpha synuclein mRNA, which has been shown to be associated with alcoholism?

► Outcome:

level of expressed alpha synuclein mRNA.

► Compared groups:

“short” vs “long” allele length
(sum score built from additive dinucleotide repeat length categorized into groups).



Challenges:

- small sample size $n = 24$ (short) + 15 (long)
- outcome not known to be normally distributed.
- aim to **confirm** that this gene is linked to alcohol dependence.

Wilcoxon-Mann-Whitney Test: motivation

Limitation of the two-sample t-test:

- Data should be **normally distributed** in each group
- **OR** the **sample size** of each group should be **large**.

Challenge:

What if we want a **reliable computation of a p-value** to compare two groups, **with small sample data not necessarily normally distributed**?

A solution:

We can use a **rank-based test**²⁰: the Wilcoxon-Mann-Whitney test²¹. It provides “exact” p-values.²²

Another advantage of Wilcoxon is its “robustness” to **outliers**, which might be convenient.

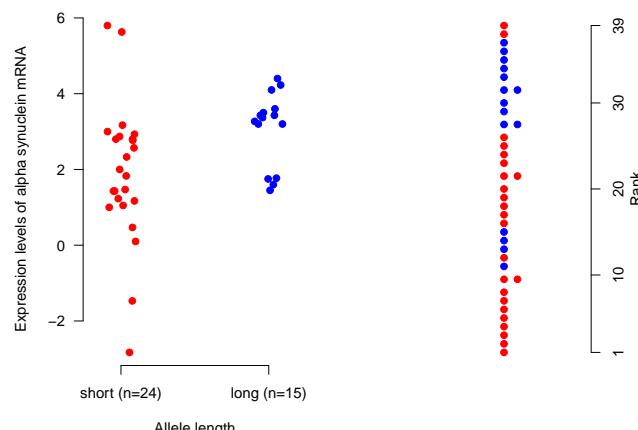
²⁰ also often called “non-parametric” test

²¹ sometimes just called “Wilcoxon” or “Mann-Whitney” test.

²² exact means that p-values are always valid (i.e. no “large n ” approximation.)

Wilcoxon test: example

p-value=0.002



Why using the ranks:

If the two groups are similar, then the ranks should be equally distributed between the two groups. **Whatever the distribution of the observations** in each group, a randomly drawn **blue observation** should be larger than a randomly drawn **red observation** in about 50% of the draws. (Here $P(X > Y) = 79.2\%$)

Wilcoxon test: practical limitation

When a significant difference is shown **we can conclude that the distribution in the two groups are different, but nothing else...** which can be **frustrating**.

Common error/overinterpretation: conclude to a difference in median.

We cannot estimate a nice matching 95% CI to quantify the “effect size”.
By contrast, to complement the p-value of a t-test we can provide a matching 95% CI of the difference in mean.

Hence unless an “exact” p-value computation is really needed, using a t-test, possibly after having transformed the data, can often be preferred²³.

²³See e.g. le Cessie, Goeman, and Dekkers. "Who is afraid of non-normal data? Choosing between parametric and non-parametric tests." European Journal of Endocrinology (2020).

