Lesson 14: Inference for difference in means from two independent samples

TB sections 5.3

Meike Niederhausen and Nicky Wakim

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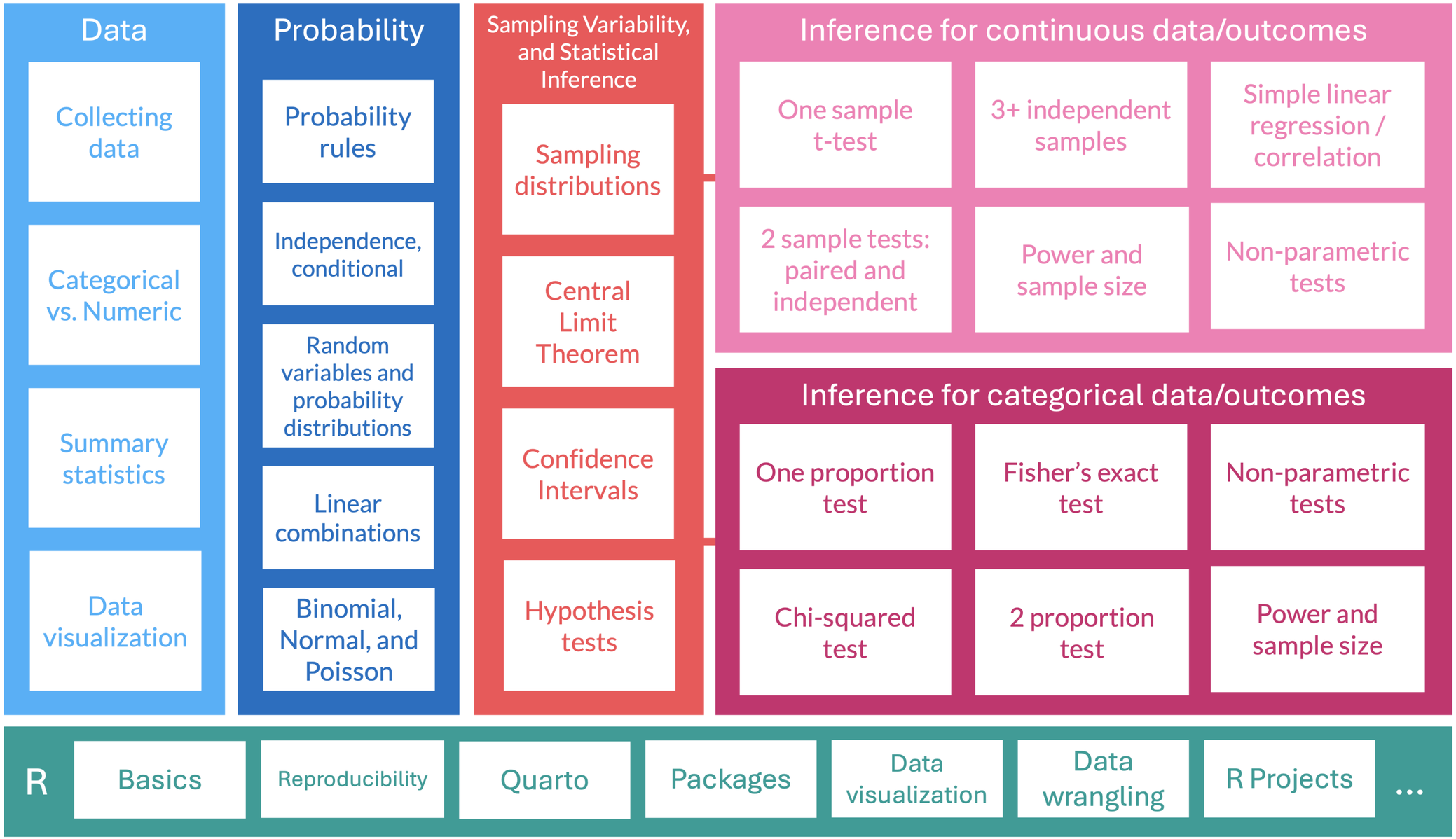
Learning Objectives

1. Identify when a research question or dataset requires two independent sample inference.
2. Construct and interpret confidence intervals for difference in means of two independent samples.
3. Run a hypothesis test for two sample independent data and interpret the results.

Where are we?


Data
Collecting data
Categorical vs. Numeric
Summary statistics
Data visualization
Probability
Probability rules
Independence, conditional
Random variables and probability distributions
Linear combinations
Binomial, Normal, and Poisson
Sampling Variability, and Statistical Inference
Sampling distributions
Central Limit Theorem
Confidence Intervals
Hypothesis tests
Inference for continuous data/outcomes
One sample t-test
3+ independent samples
Simple linear regression / correlation
2 sample tests: paired and independent
Power and sample size
Non-parametric tests
Inference for categorical data/outcomes
One proportion test
Fisher's exact test
Non-parametric tests
Chi-squared test
2 proportion test
Power and sample size
R
Basics
Reproducibility
Quarto
Packages
Data visualization
Data wrangling
R Projects
...

Different types of inference based on different data types

Lesson	Section	Population parameter	Symbol (pop)	Point estimate	Symbol (sample)	SE
11	5.1	Pop mean	μ	Sample mean	$\overline{x}$	$\frac{s}{\sqrt{n}}$
12	5.2	Pop mean of paired diff	μ_d or δ	Sample mean of paired diff	$\overline{x}_d$	$\frac{s_d}{\sqrt{n}}$
13	5.3	Diff in pop means	$\mu_1 - \mu_2$	Diff in sample means	$\overline{x}_1 - \overline{x}_2$	**????**
15	8.1	Pop proportion	p	Sample prop	$\widehat{p}$	
15	8.2	Diff in pop prop's	$p_1 - p_2$	Diff in sample prop's	$\widehat{p}_1 - \widehat{p}_2$	

Learning Objectives

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What are data from two independent sample?

- **Two independent samples:** Individuals between and within samples are independent
  - Typically: measure the same outcome for each sample, but typically the two samples differ based on a single variable

- Examples
  - Any study where participants are randomized to a control and treatment group
  - Study with two groups based on their exposure to some condition (can be observational)
  - Book: “Does treatment using embryonic stem cells (ESCs) help improve heart function following a heart attack?”
  - Book: “Is there evidence that newborns from mothers who smoke have a different average birth weight than newborns from mothers who do not smoke?”

- Pairing (like comparing before and after) may not be feasible

Poll Everywhere Question 1

For two independent samples: Population parameters vs. sample statistics

Population parameter

- Population 1 mean: μ_1
- Population 2 mean: μ_2

- Difference in means: $\mu_1 - \mu_2$

- Population 1 standard deviation: σ_1
- Population 2 standard deviation: σ_2

Sample statistic (point estimate)

- Sample 1 mean: $\overline{x}_1$
- Sample 2 mean: $\overline{x}_2$

- Difference in sample means: $\overline{x}_1 - \overline{x}_2$

- Sample 1 standard deviation: s_1
- Sample 2 standard deviation: s_2

Does caffeine increase finger taps/min (on average)?

- Use this example to illustrate how to calculate a confidence interval and perform a hypothesis test for two independent samples

Study Design:[1]

- 70 college students students were trained to tap their fingers at a rapid rate
- Each then drank 2 cups of coffee (double-blind)
  - **Control** group: decaf
  - **Caffeine** group: ~ 200 mg caffeine
- After 2 hours, students were tested.
- **Taps/minute** recorded

Does caffeine increase finger taps/min (on average)?

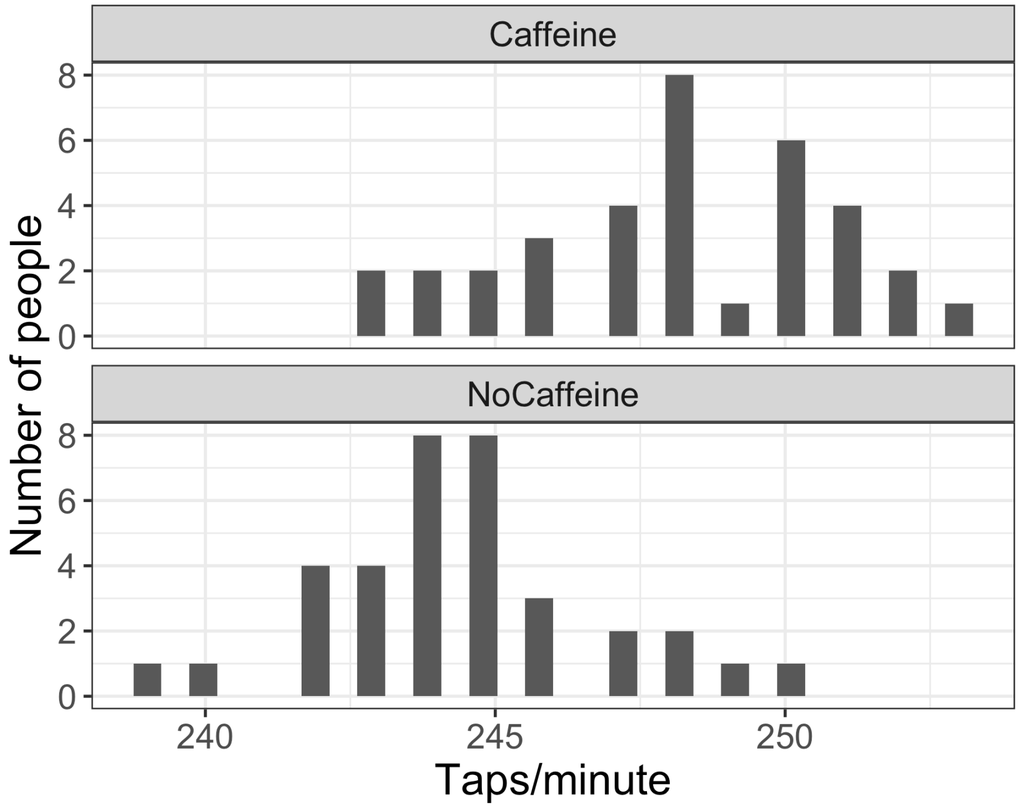
- Load the data from the csv file `CaffeineTaps.csv`
- The code below is for when the data file is in a folder called `data` that is in your R project folder (your working directory)

```
1 CaffTaps <- read.csv(here::here("data", "CaffeineTaps_n35.csv"))
2
3 glimpse(CaffTaps)
```

```
Rows: 70
Columns: 2
$ Taps  <int> 246, 248, 250, 252, 248, 250, 246, 248, 245, 250, 242, 245, 244,…
$ Group <chr> "Caffeine", "Caffeine", "Caffeine", "Caffeine", "Caffeine", "Caf…
```

EDA: Explore the finger taps data

- Code to make these histograms



- Summary statistics stratified by group

Group	variable	n	mean	sd
Caffeine	Taps	35	248.114	2.621
NoCaffeine	Taps	35	244.514	2.318

Then calculate the difference between the means:

```
1 diff(sumstats$mean)
```

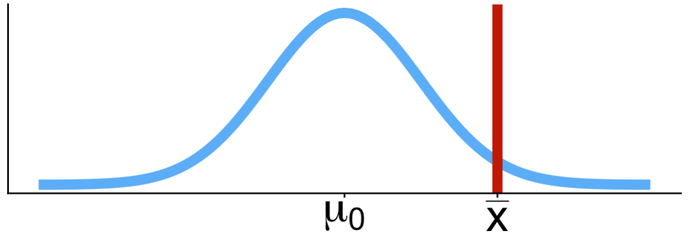
```
[1] -3.6
```

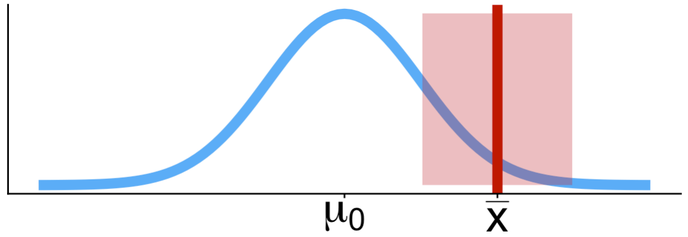
- Note that we cannot calculate 35 differences in taps because these data are not paired!!
- Different individuals receive caffeine vs. do not receive caffeine

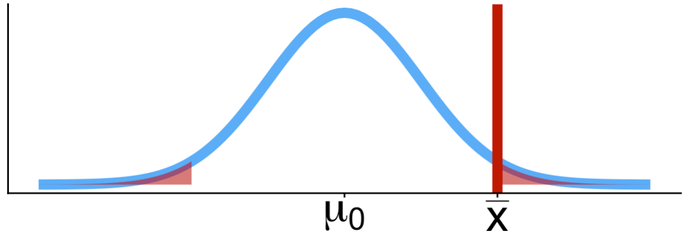
Poll Everywhere Question 2

What would the distribution look like for 2 independent samples?

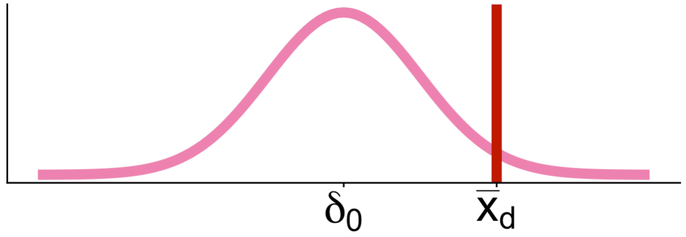
Single-sample mean:

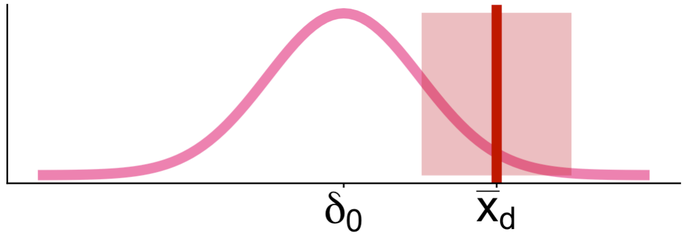


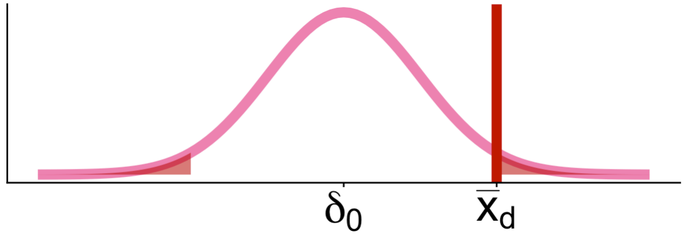




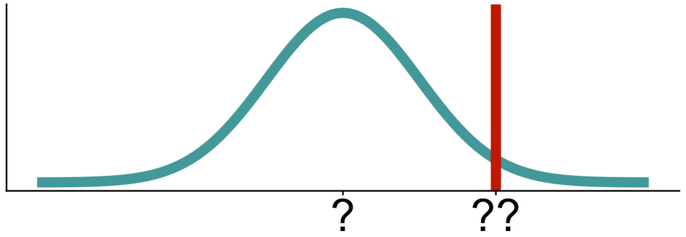
Paired mean difference:

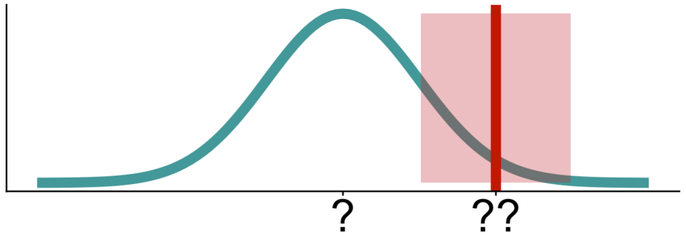


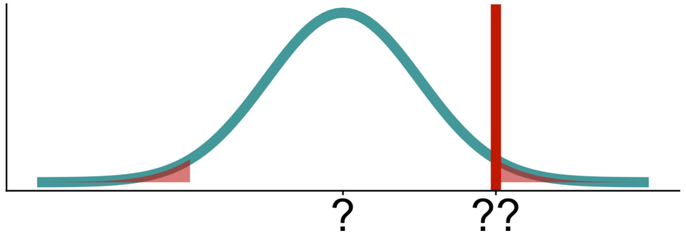




Diff in means of 2 ind samples:







What distribution does $\overline{X}_1 - \overline{X}_2$ have? (when we know pop sd's)

- Let $\overline{X}_1$ and $\overline{X}_2$ be the means of random samples from two independent groups, with parameters shown in table:
- Some theoretical statistics:
  - If $\overline{X}_1$ and $\overline{X}_2$ are independent normal RVs, then $\overline{X}_1 - \overline{X}_2$ is also normal
  - What is the mean of $\overline{X}_1 - \overline{X}_2$?

$$E[\overline{X}_1 - \overline{X}_2] = E[\overline{X}_1] - E[\overline{X}_2] = \mu_1 - \mu_2$$

  - What is the standard deviation of $\overline{X}_1 - \overline{X}_2$?

$$Var(\overline{X}_1 - \overline{X}_2) = Var(\overline{X}_1) + Var(\overline{X}_2) = \frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}$$

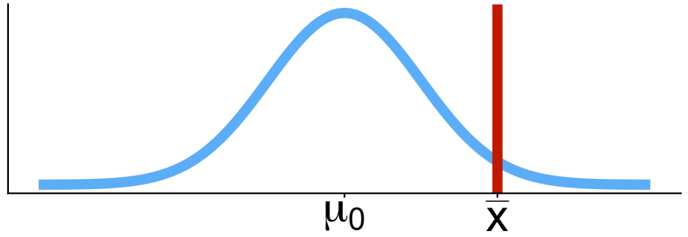
$$SD(\overline{X}_1 - \overline{X}_2) = \sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}$$

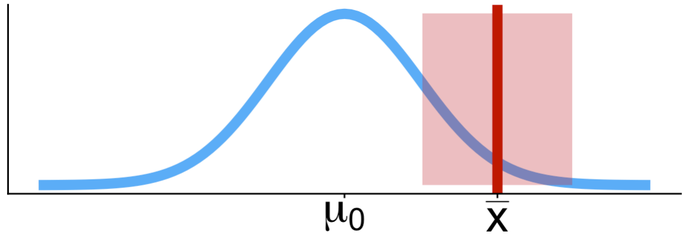
	Gp 1	Gp 2
sample size	n_1	n_2
pop mean	μ_1	μ_2
pop sd	σ_1	σ_2

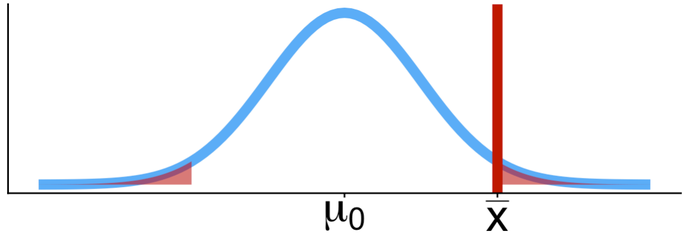
$$\overline{X}_1 - \overline{X}_2 \sim$$

What would the distribution look like for 2 independent samples?

Single-sample mean:







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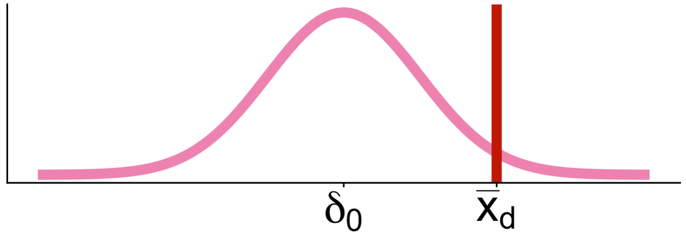

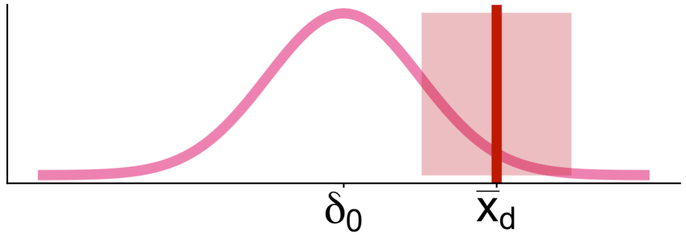

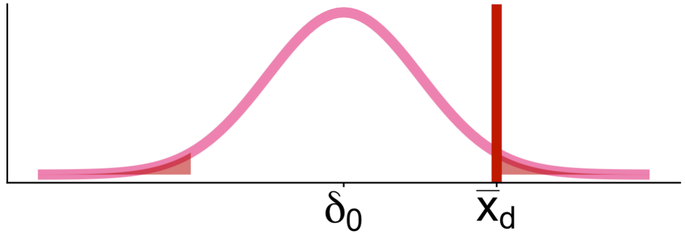

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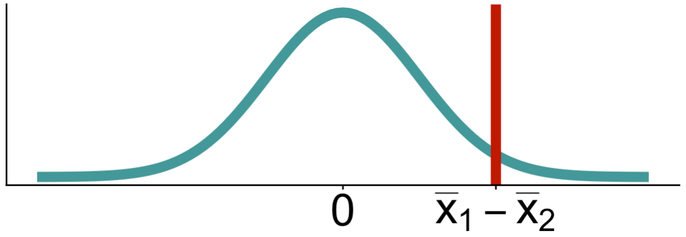
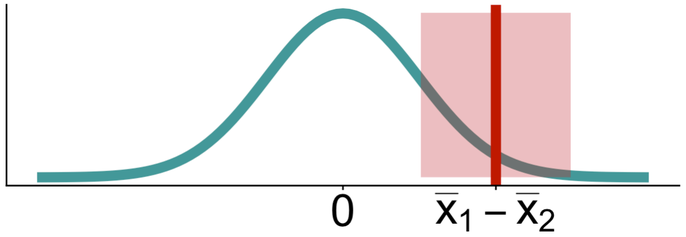
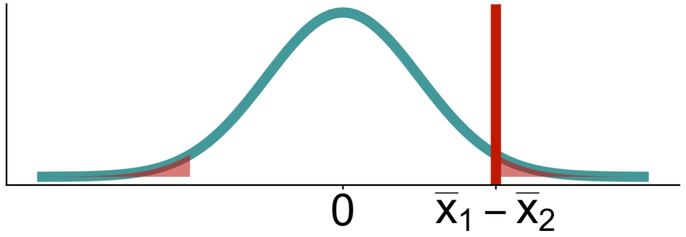


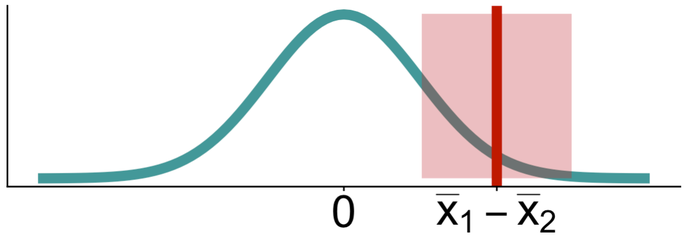




Approaches to answer a research question

- **Research question is a generic form for 2 independent samples:** Is there evidence to support that the population means are different from each other?

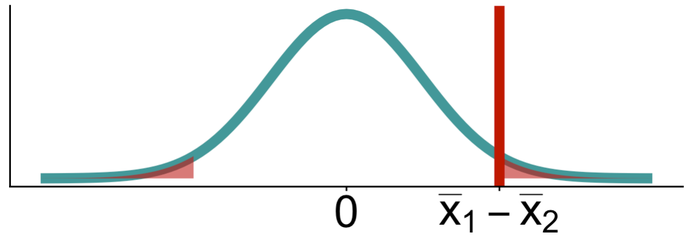
Calculate **CI for the mean difference** $\mu_1 - \mu_2$:



$$\bar{x}_1 - \bar{x}_2 \pm t^* \times \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

- with t^* = t-score that aligns with specific confidence interval

Run a **hypothesis test:**



Hypotheses

$$H_0 : \mu_1 = \mu_2$$
$$H_A : \mu_1 \neq \mu_2$$
$$(or \ <, >)$$

Test statistic

$$t_{\bar{x}_1 - \bar{x}_2} = \frac{\bar{x}_1 - \bar{x}_2 - 0}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

Learning Objectives

1. Identify when a research question or dataset requires two independent sample inference.

2. Construct and interpret confidence intervals for difference in means of two independent samples.

3. Run a hypothesis test for two sample independent data and interpret the results.

95% CI for the difference in population mean taps $\mu_1 - \mu_2$

Confidence interval for $\mu_1 - \mu_2$

$$\overline{x}_1 - \overline{x}_2 \pm t^* \times \text{SE}$$

- with $\text{SE} = \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$ if population sd is not known

- t^* depends on the confidence level and degrees of freedom
  - degrees of freedom (df) is: $df = n_1 + n_2 - 2$

95% CI for the difference in population mean taps

```
1 CaffTaps %>% group_by(Group) %>% get_summary_stats(type = "mean_sd") %>%
2   gt() %>% tab_options(table.font.size = 40)
```

Group	variable	n	mean	sd
Caffeine	Taps	35	248.114	2.621
NoCaffeine	Taps	35	244.514	2.318

95% CI for $\mu_{caff} - \mu_{ctrl}$:

$$\overline{x}_{\text{caff}} - \overline{x}_{\text{ctrl}} \pm t^* \cdot \sqrt{\frac{s^2_{\text{caff}}}{n_{\text{caff}}} + \frac{s^2_{\text{ctrl}}}{n_{\text{ctrl}}}}$$

$$248.114 - 244.514 \pm 1.995 \cdot \sqrt{\frac{2.621^2}{35} + \frac{2.318^2}{35}}$$

$$3.6 \pm 1.995 \cdot \sqrt{0.196 + 0.154}$$

$$(2.42, 4.78)$$

Used t^* = `qt(0.975, df=68)` = 1.995

Conclusion:
We are 95% confident that the difference in (population) mean finger taps/min between the caffeine and control groups is between 2.42 mg/dL and 4.78 mg/dL.

95% CI for the difference in population mean taps (using R)

```r
t.test(formula = Taps ~ Group, data = CaffTaps, var.equal = T)
```

```
    Two Sample t-test

data:  Taps by Group
t = 6.0867, df = 68, p-value = 5.996e-08
alternative hypothesis: true difference in means between group Caffeine and group
NoCaffeine is not equal to 0
95 percent confidence interval:
 2.419768 4.780232
sample estimates:
  mean in group Caffeine mean in group NoCaffeine
                248.1143                 244.5143
```

- We can tidy the output

estimate	estimate1	estimate2	statistic	p.value	parameter	conf.low	conf.high	method	alternative
3.6	248.1143	244.5143	6.086677	6.265631e-08	67.00222	2.41945	4.78055	Welch Two Sample t-test	two.sided

Conclusion:

We are 95% confident that the difference in (population) mean finger taps/min between the caffeine and control groups is between 2.419 mg/dL and 4.781 mg/dL.

Poll Everywhere Question 3

Learning Objectives

1. Identify when a research question or dataset requires two independent sample inference.
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Reference: Steps in a Hypothesis Test

1. Check the **assumptions**
2. Set the **level of significance** α
3. Specify the **null** (H_0) and **alternative** (H_A) **hypotheses**
    1. In symbols
    2. In words
    3. Alternative: one- or two-sided?
4. Calculate the **test statistic**.
5. Calculate the **p-value** based on the observed test statistic and its sampling distribution
6. Write a **conclusion** to the hypothesis test
    1. Do we reject or fail to reject H_0?
    2. Write a conclusion in the context of the problem

Step 1: Check the assumptions

- The assumptions to run a hypothesis test on a sample are:
  - **Independent observations**: Each observation from both samples is independent from all other observations
  - **Approximately normal sample or big n**: the distribution of *each sample* should be approximately normal, *or* the sample size of *each sample* should be at least 30

- These are the criteria for the Central Limit Theorem in Lesson 09: Variability in estimates

- In our example, we would check the assumptions with a statement:
  - The observations are independent from each other. Each caffeine group (aka sample) has 35 individuals. Thus, we can use CLT to approximate the sampling distribution for each sample.

Step 2: Set the level of significance

- **Before doing a hypothesis test**, we set a cut-off for how small the p-value should be in order to reject H_0.
- Typically choose $\alpha = 0.05$

- See Lesson 11: Hypothesis Testing 1: Single-sample mean

Step 3: Null & Alternative Hypotheses

Notation for hypotheses (for two ind samples)

$$H_0 : \mu_1 = \mu_2$$
$$\text{vs. } H_A : \mu_1 \neq, <, \text{or}, > \mu_2$$

Hypotheses test for example

$$H_0 : \mu_{caff} = \mu_{ctrl}$$
$$\text{vs. } H_A : \mu_{caff} > \mu_{ctrl}$$

- Under the null hypothesis: $\mu_1 = \mu_2$, so the difference in the means is $\mu_1 - \mu_2 = 0$

$H_A : \mu_1 \neq \mu_2$

- not choosing a priori whether we believe the population mean of group 1 is different than the population mean of group 2

$H_A : \mu_1 < \mu_2$

- believe that population mean of group 1 is less than population mean of group 2

$H_A : \mu_1 > \mu_2$

- believe that population mean of group 1 is greater than population mean of group 2

- $H_A : \mu_1 \neq \mu_2$ is the most common option, since it's the most conservative

Step 3: Null & Alternative Hypotheses: another way to write it

- Under the null hypothesis: $\mu_1 = \mu_2$, so the difference in the means is $\mu_1 - \mu_2 = 0$

$H_A : \mu_1 \neq \mu_2$

- not choosing a priori whether we believe the population mean of group 1 is different than the population mean of group 2

$H_A : \mu_1 > \mu_2$

- believe that population mean of group 1 is greater than population mean of group 2

$H_A : \mu_1 < \mu_2$

- believe that population mean of group 1 is less than population mean of group 2

$H_A : \mu_1 - \mu_2 \neq 0$

- not choosing a priori whether we believe the difference in population means is greater or less than 0

$H_A : \mu_1 - \mu_2 > 0$

- believe that difference in population means (mean 1 - mean 2) is greater than 0

$H_A : \mu_1 - \mu_2 < 0$

- believe that difference in population means (mean 1 - mean 2) is less than 0

Step 3: Null & Alternative Hypotheses

- **Question**: Is there evidence to support that drinking caffeine increases the number of finger taps/min?

Null and alternative hypotheses in **words**

- H_0: The population difference in mean finger taps/min between the caffeine and control groups is 0
- H_A: The population difference in mean finger taps/min between the caffeine and control groups is greater than 0

Null and alternative hypotheses in **symbols**

$$H_0 : \mu_{caff} - \mu_{ctrl} = 0$$
$$H_A : \mu_{caff} - \mu_{ctrl} > 0$$

Step 4: Test statistic

Recall, for a two sample independent means test, we have the following test statistic:

$$t_{\overline{x}_1-\overline{x}_2} = \frac{\overline{x}_1 - \overline{x}_2 - 0}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

- $\overline{x}_1, \overline{x}_2$ are the sample means
- $\mu_0 = 0$ is the mean value specified in H_0
- s_1, s_2 are the sample SD's
- n_1, n_2 are the sample sizes

- Statistical theory tells us that $t_{\overline{x}_1-\overline{x}_2}$ follows a **student's t-distribution** with
  - $df = n_1 + n_2 - 2$

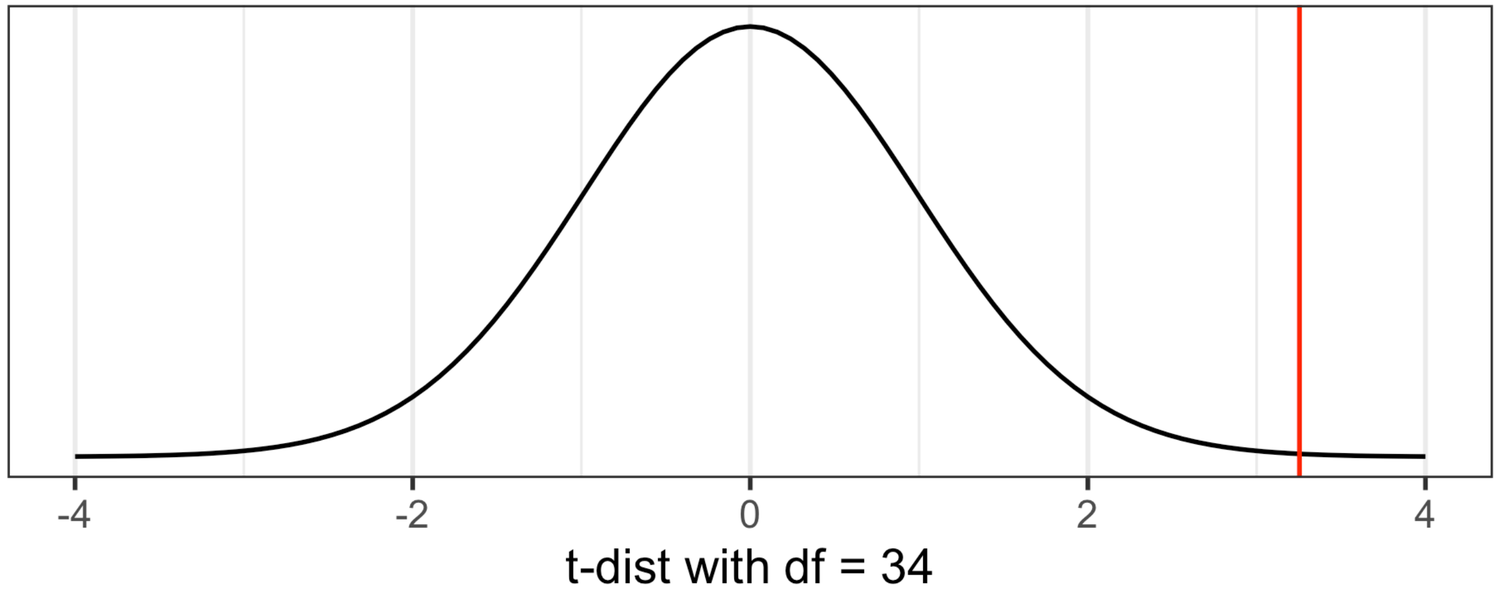
Step 4: Test statistic (where we do not know population sd)

From our example: Recall that $\overline{x}_1 = 248.114$, $s_1 = 2.621$, $n_1 = 35$, $\overline{x}_2 = 244.514$, $s_2 = 2.318$, and $n_2 = 35$:

The test statistic is:

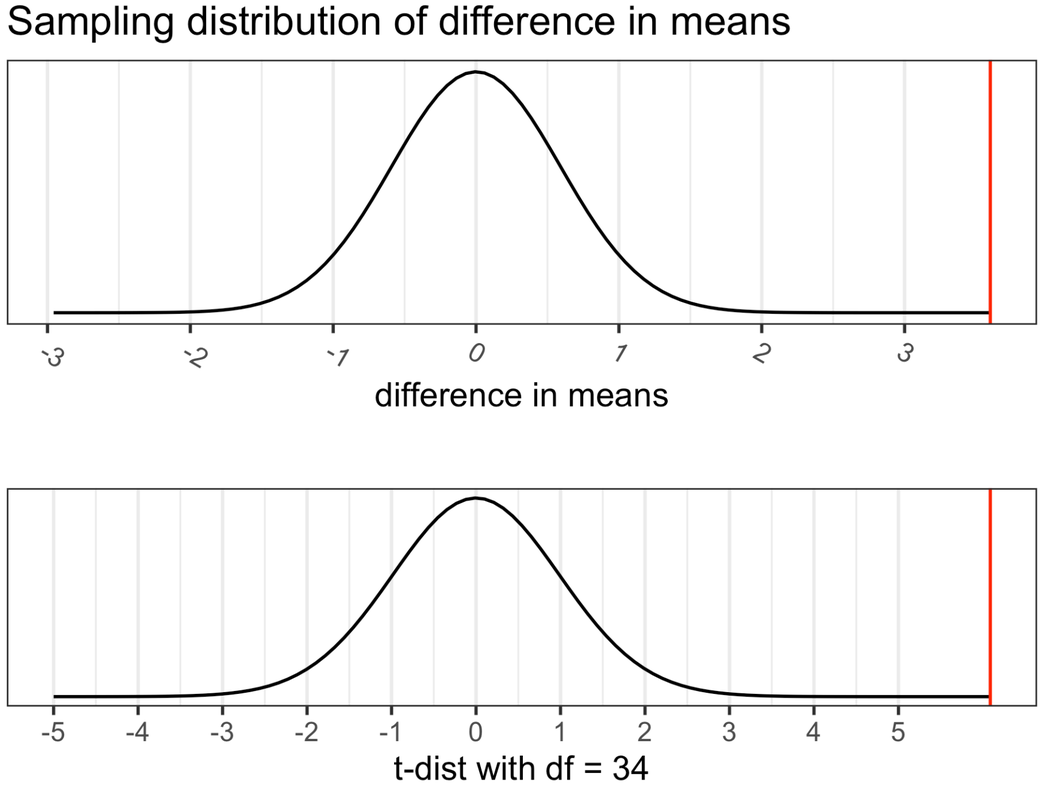
$$\text{test statistic} = t_{\overline{x}_1 - \overline{x}_2} = \frac{\overline{x}_1 - \overline{x}_2 - 0}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} = \frac{248.114 - 244.514 - 0}{\sqrt{\frac{2.621^2}{35} + \frac{2.318^2}{35}}} = 6.0869$$

- Statistical theory tells us that $t_{\overline{x}_1 - \overline{x}_2}$ follows a **Student's t-distribution** with $df = n_1 + n_2 - 2 = 68$



Step 5: p-value

The **p-value** is the **probability** of obtaining a test statistic *just as extreme or more extreme* than the observed test statistic assuming the null hypothesis H_0 is true.



Calculate the *p*-value using the **Student's t-distribution** with $df = n_1 + n_2 - 2 = 68$:

$$\begin{aligned} \text{p-value} &= P(T > 6.08691) \\ &= 3 \times 10^{-8} \end{aligned}$$

```r
pt(tstat,
   df = 68,
   lower.tail = FALSE)
```

```
[1] 2.99498e-08
```

Step 4-5: test statistic and p-value together using `t.test()`

- I will have reference slides at the end of this lesson to show other options and how to "tidy" the results

```
1 t.test(formula = Taps ~ Group, alternative = "greater", data = CaffTaps)
```

```
    Welch Two Sample t-test

data:  Taps by Group
t = 6.0867, df = 67.002, p-value = 3.133e-08
alternative hypothesis: true difference in means between group Caffeine and group
NoCaffeine is greater than 0
95 percent confidence interval:
 2.613502      Inf
sample estimates:
  mean in group Caffeine mean in group NoCaffeine
                 248.1143                 244.5143
```

- Why are the degrees of freedom different? (see Slide Section 5.4)
  - Degrees of freedom in R is more accurate
  - Using our approximation in our calculation is okay, but conservative

Poll Everywhere Question 4

Step 6: Conclusion to hypothesis test

$$H_0 : \mu_1 = \mu_2$$
$$\text{vs. } H_A : \mu_1 > \mu_2$$

- Need to compare p-value to our selected $\alpha = 0.05$
- Do we reject or fail to reject H_0?

If $\text{p-value} < \alpha$, reject the null hypothesis

- There is sufficient evidence that the difference in population means is discernibly greater than 0 (p-value = ___)

If $\text{p-value} \geq \alpha$, fail to reject the null hypothesis

- There is insufficient evidence that the difference in population means is discernibly greater than 0 (p-value = ___)

Step 6: Conclusion to hypothesis test

$$H_0 : \mu_{caff} - \mu_{ctrl} = 0$$
$$H_A : \mu_{caff} - \mu_{ctrl} > 0$$

- Recall the p-value = 3×10^{-8}
- Use α = 0.05.
- Do we reject or fail to reject H_0?

Conclusion statement:

- Stats class conclusion
  - There is sufficient evidence that the (population) difference in mean finger taps/min with vs. without caffeine is greater than 0 (p-value < 0.001).
- More realistic manuscript conclusion:
  - The mean finger taps/min were 248.114 (SD = 2.621) and 244.514 (SD = 2.318) for the control and caffeine groups, and the increase of 3.6 taps/min was statistically discrenible (p-value < 0.001).

Reference: Ways to run a 2-sample t-test in R

R: 2-sample t-test (with long data)

- The `CaffTaps` data are in a *long* format, meaning that
  - all of the outcome values are in one column and
  - another column indicates which group the values are from
- This is a common format for data from multiple samples, especially if the sample sizes are different.

```
(Taps_2ttest <- t.test(formula = Taps ~ Group,
                       alternative = "greater",
                       data = CaffTaps))
```

```
    Welch Two Sample t-test

data:  Taps by Group
t = 6.0867, df = 67.002, p-value = 3.133e-08
alternative hypothesis: true difference in means between group Caffeine and group
NoCaffeine is greater than 0
95 percent confidence interval:
 2.613502      Inf
sample estimates:
  mean in group Caffeine mean in group NoCaffeine
                248.1143                   244.5143
```

`tidy` the `t.test` output

```
# use tidy command from broom package for briefer output that's a tibble
tidy(Taps_2ttest) %>% gt() %>% tab_options(table.font.size = 40)
```

estimate	estimate1	estimate2	statistic	p.value	parameter	conf.low	conf.high	method	alternative
3.6	248.1143	244.5143	6.086677	3.132816e-08	67.00222	2.613502	Inf	Welch Two Sample t-test	greater

- Pull the p-value:

```
tidy(Taps_2ttest)$p.value  # we can pull specific values from the tidy output
```

```
[1] 3.132816e-08
```

R: 2-sample t-test (with wide data)

```r
# make CaffTaps data wide: pivot_wider needs an ID column so that it
# knows how to "match" values from the Caffeine and NoCaffeine groups
CaffTaps_wide <- CaffTaps %>%
  mutate(id = c(rep(1:10, 2), rep(11:35, 2))) %>% #  "fake" IDs for pivot_wid
  pivot_wider(names_from = "Group",
              values_from = "Taps")

glimpse(CaffTaps_wide)
```

```
Rows: 35
Columns: 3
$ id         <int> 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, …
$ Caffeine   <int> 246, 248, 250, 252, 248, 250, 246, 248, 245, 250, 251, 251,…
$ NoCaffeine <int> 242, 245, 244, 248, 247, 248, 242, 244, 246, 242, 244, 245,…
```

```r
t.test(x = CaffTaps_wide$Caffeine, y = CaffTaps_wide$NoCaffeine, alternative =
  tidy() %>% gt() %>% tab_options(table.font.size = 40)
```

estimate	estimate1	estimate2	statistic	p.value	parameter	conf.low	conf.high	method	alternative
3.6	248.1143	244.5143	6.086677	3.132816e-08	67.00222	2.613502	Inf	Welch Two Sample t-test	greater

Why are the df's in the R output different?

From many slides ago:

- Statistical theory tells us that $t_{\overline{x}_1 - \overline{x}_2}$ follows a **student's t-distribution** with
  - $df = n_1 + n_2 - 2$

The actual degrees of freedom are calculated using Satterthwaite's method for the one-sided tests is:

$$\nu = \frac{[(s_1^2/n_1) + (s_2^2/n_2)]^2}{(s_1^2/n_1)^2/(n_1 - 1) + (s_2^2/n_2)^2/(n_2 - 1)} = \frac{[SE_1^2 + SE_2^2]^2}{SE_1^4/df_1 + SE_2^4/df_2}$$

Verify the *p*-value in the R output using ν = 17.89012:

```
1 pt(3.3942, df = 17.89012, lower.tail = FALSE)
```

```
[1] 0.001627588
```