Hypothesis testing: one sample

Is P53 gene expressed at a lower level in cancer patients than in healthy people?

- We are interested if a P53 gene expression is **lowered** in population of cancer patients compared to the healthy population.
- We know that mean gene expression in the healthy population is μ_h=50 mRNAs/cell We are interested in deciding whether or not the mean expression in cancer population is **lower than** in healthy population. It is called hypothesis H_1. Here H_1 is **one-sided**. If we asked: cancer not equal healthy – two-sided hypothesis
- Assume we have a sample of 100 cancer patients with sample mean $\bar{X}$ =48 mRNAs/cell and sample standard deviation S=10 mRNA/cell
- **Based on this sample can we reject the ``strawman'' or null hypothesis H_0:** cancer = healthy and accept **alternative hypothesis H_1 that P53 in cancer is lower than in healthy?**

Two types of errors

True State of Nature

Decision Made		H_0 is true	H_a is true
	Accept H_0	**Correct decision** Probability = $1 - \alpha$	**Type II error** Probability = β
	Reject H_0	**Type I error** Probability = α (significance level)	**Correct decision** Probability = $1 - \beta$ (power)

P-Values of Hypothesis Tests

- P-value: what is the probability to get the observed sample mean of $\bar{X}$ =48 mRNAs/cell (or even smaller) in a healthy population with μ_h=50 mRNAs/cell

- If P-value is small – the null hypothesis is likely wrong and thus my probability of making a type I error (incorrectly rejecting a null hypothesis) is small

- P-value answers the question: if I reject a null hypothesis H_0 based on the sample, what is the probability that I am making a type I error?

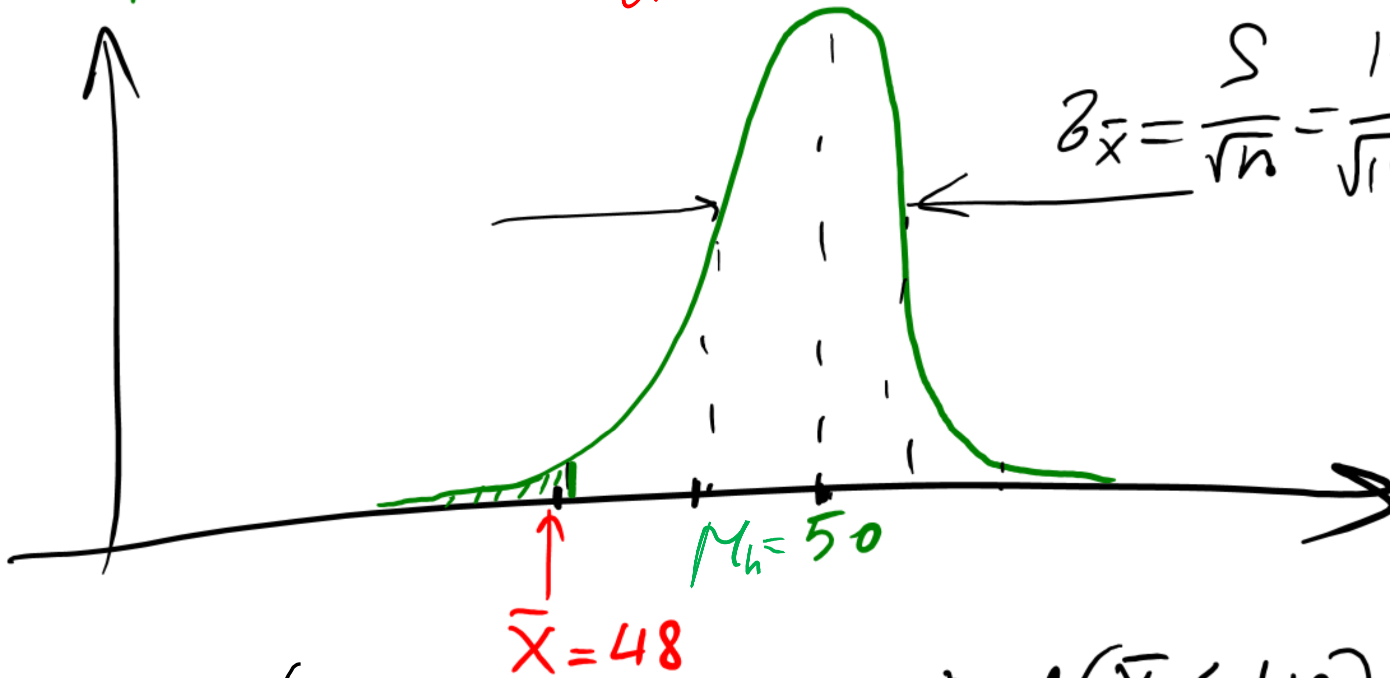
$\mu_h = 50$

$n = 100, \quad \bar{X} = 48, \quad S = 10$

$H_0: \mu_c = \mu_h$

One-sided hypothesis $H_1: \mu_c < \mu_h$

$$\sigma_{\bar{X}} = \frac{S}{\sqrt{n}} = \frac{10}{\sqrt{100}} = 1$$



P-Value $(\bar{X} = 48 \mid H_0: \mu_c = \mu_h) = \text{Prob}(\bar{X} \leq 48) = 2.5\% = 0.025$

If $H_1: \mu_c \neq \mu_h$: p-value $= \text{Prob}(\bar{X} \leq 48) + \text{Prob}(\bar{X} \geq 52) = 0.05$

Two-sided hypothesis

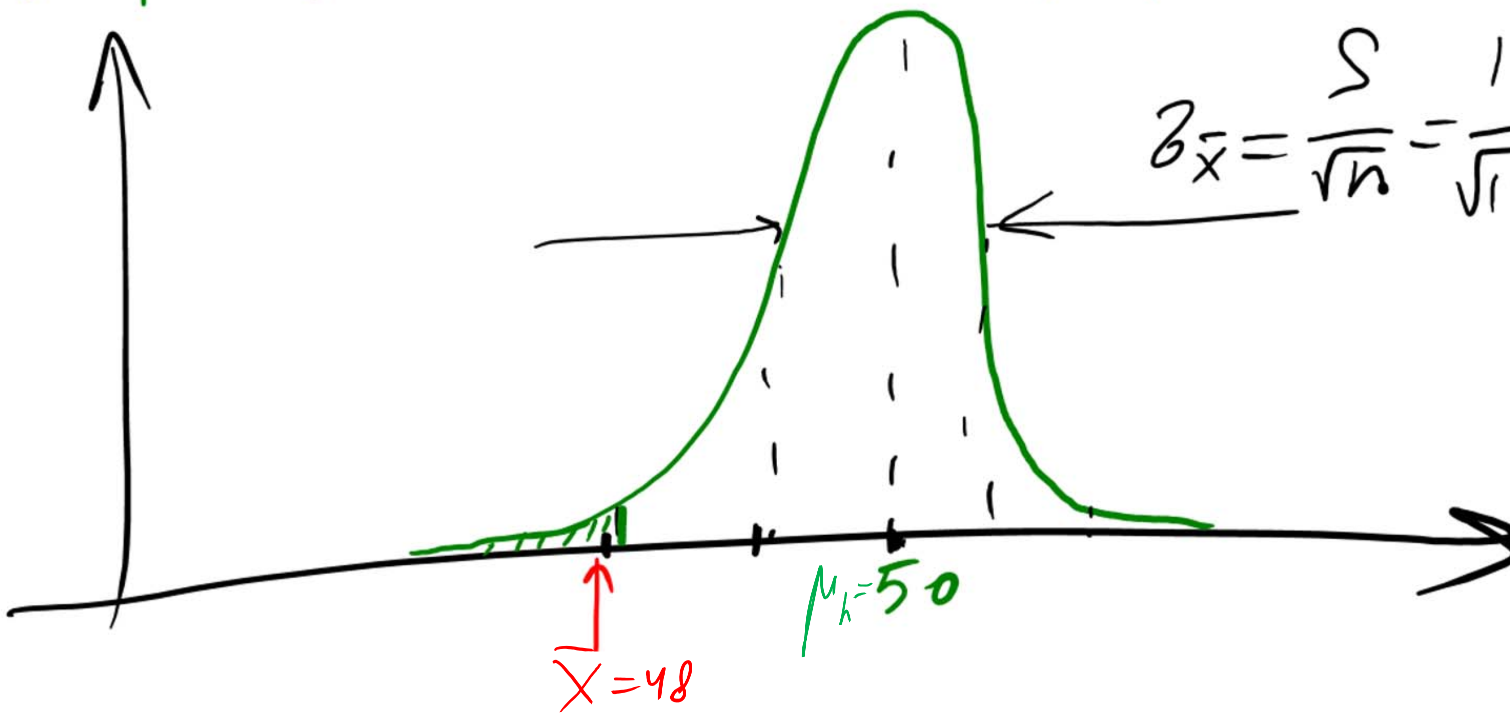
$\mu_h = 50$

$H_0: \mu_c = \mu_h$

$n = 100, \quad \bar{X} = 48, \quad s = 10$

$H_1: \mu_c < \mu_h$

$\sigma_{\bar{X}} = \frac{s}{\sqrt{n}} = \frac{10}{\sqrt{100}} = 1$

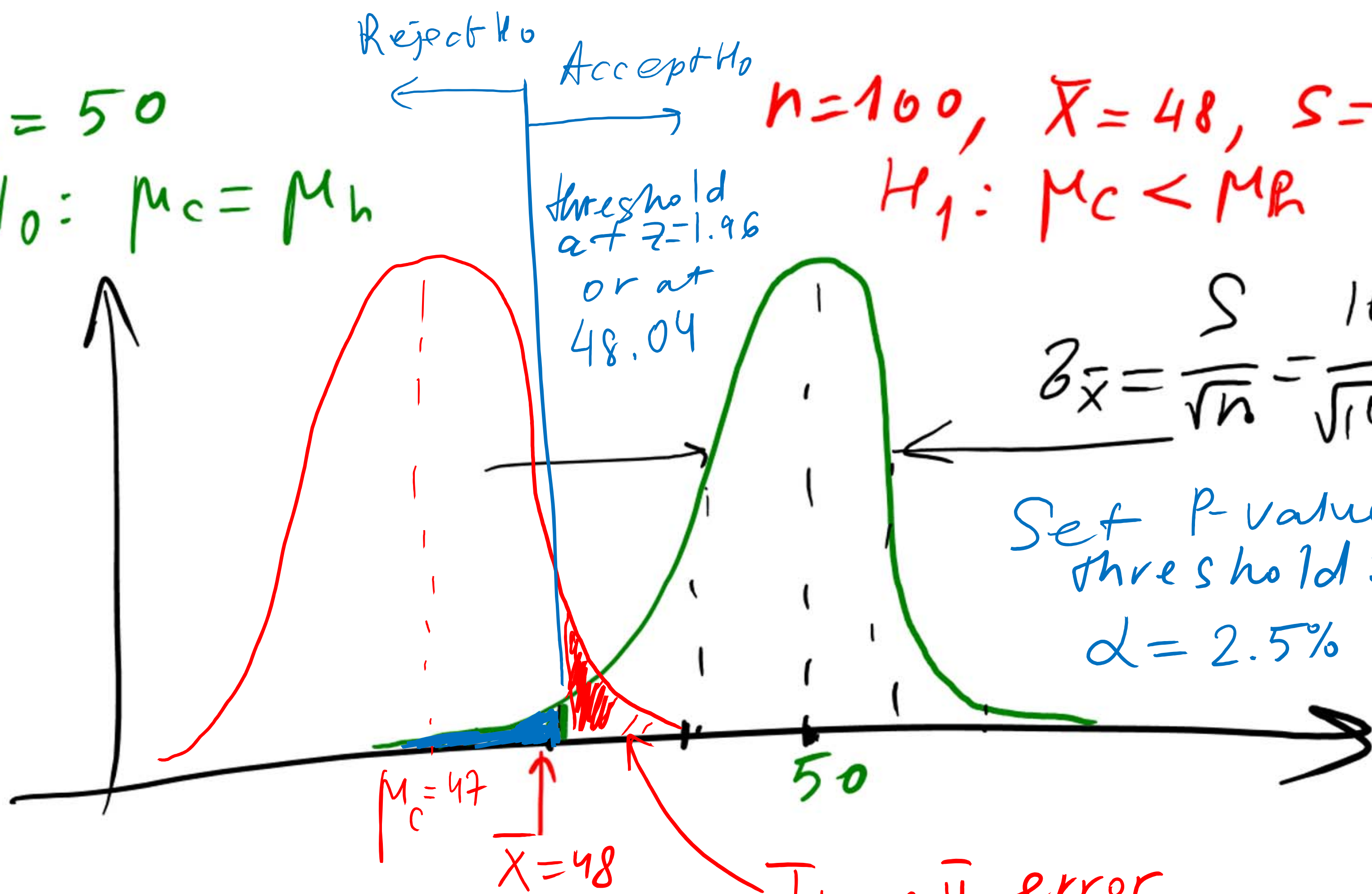


$\mu_h = 50$

$H_0: \mu_c = \mu_h$

$n = 100, \quad \bar{X} = 48, \quad s = 10$

$H_1: \mu_c < \mu_h$



$$\sigma_{\bar{x}} = \frac{s}{\sqrt{n}} = \frac{10}{\sqrt{100}} = 1$$

Set p-value threshold: $\alpha = 2.5\%$

$$\beta = P(\text{Accept } H_0 \mid H_1 \text{ is true}) = \int_{48.04}^{\infty} \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{(x-47)^2}{2}\right) dx =$$

$$= 1 - \Phi(1.04) = 15\%$$

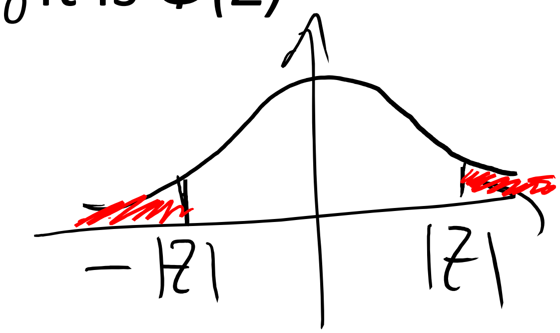
$$\alpha = 1 - \Phi(1.96) = 2.5\%$$

Generalizations

- What if H_1 is a two-sided hypothesis?
- A: P-value is $2(1-\Phi(|Z|))$, where $Z=(\bar{X}-\mu_0)/[S/\sqrt{n}]$

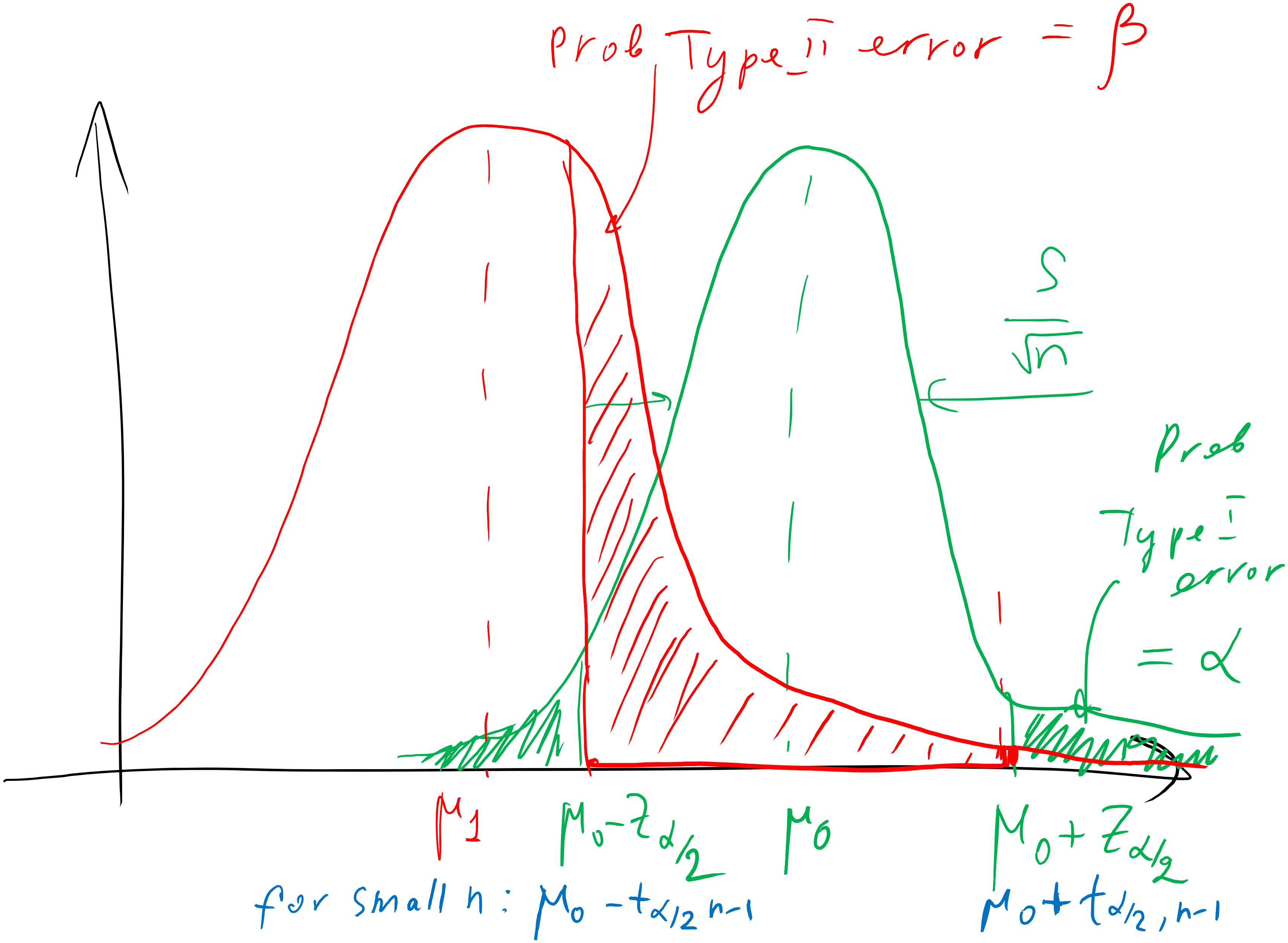
  Compare to: For one sized $\mu_1 > \mu_0$ it is $1-\Phi(Z)$
  For one sized $\mu_1 < \mu_0$ it is $\Phi(Z)$
- If α is given use $\mu_0 +/- z_{\alpha/2}$ as thresholds

to reject the null hypothesis



- What if the sample size n is small (say n<10):
- A: Use t-distribution with n-1 degrees of freedom for 2-sided $P\text{-}value=2(1-CDF\_Tdist(|T|))$ where $T=(\bar{X}-\mu_0)/[S/\sqrt{n}]$.
- For given α use $\mu_0 +/- t_{\alpha/2,n-1}$ to reject the null hypothesis

Type II error for two-sided hypothesis



Type II Error and Choice of Sample Size

Assume you know the minimum $\delta = |\mu_1 - \mu_0|$ that you care about.
What is the minimal sample you should use to separate H0 and H1 hypotheses if your tolerance to type I and type II errors is α and β ?

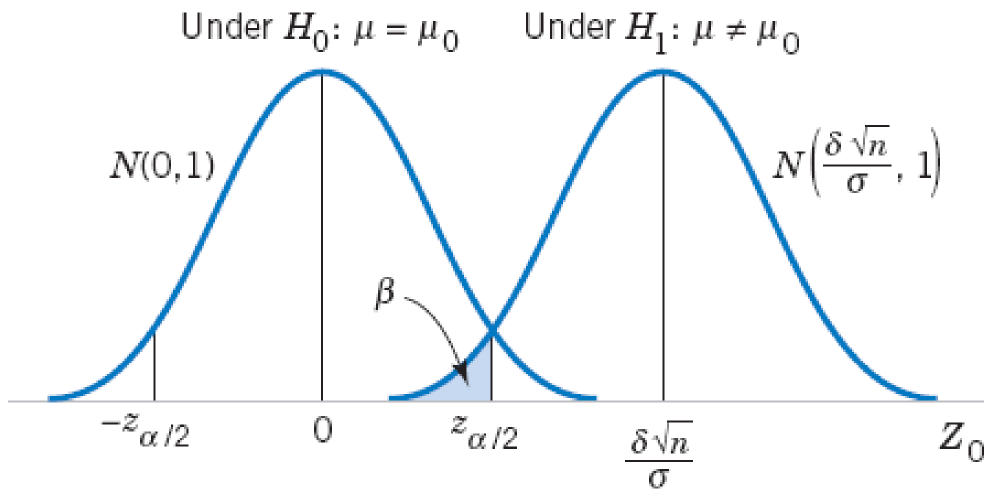
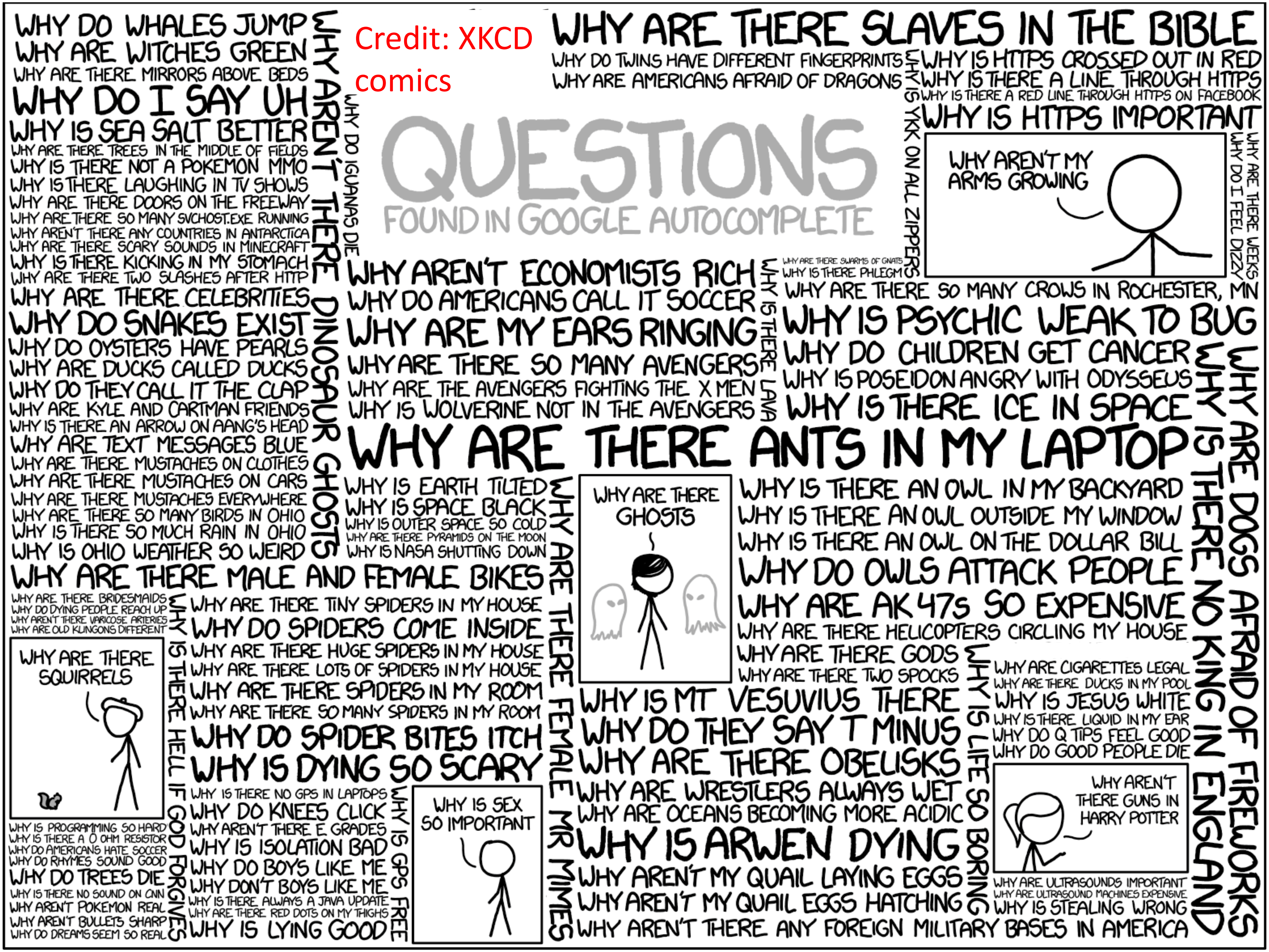


Figure 9-9 The distribution of Z_0 under H_0 and H_1.

$$\frac{\delta\sqrt{n}}{\sigma} = z_{\alpha/2} + z_\beta$$

$$n \simeq \frac{(z_{\alpha/2} + z_\beta)^2 \sigma^2}{\delta^2} \quad \text{where} \quad \delta = \mu - \mu_0 \qquad (9\text{-}22)$$



Credit: XKCD comics

P-value vs α

- P-value gives the result of accept/reject the null hypothesis at all values of α

Table 11.1: A commonly adopted convention for reporting p values: in many places it is conventional to report one of four different things (e.g., $p < .05$) as shown below. I've included the "significance stars" notation (i.e., a * indicates $p < .05$) because you sometimes see this notation produced by statistical software. It's also worth noting that some people will write *n.s.* (not significant) rather than $p > .05$.

Usual notation	Signif. stars	English translation	The null is...
$p > .05$		The test wasn't significant	Retained
$p < .05$	*	The test was significant at $\alpha = .05$ but not at $\alpha = .01$ or $\alpha = .001$.	Rejected
$p < .01$	**	The test was significant at $\alpha = .05$ and $\alpha = .01$ but not at $\alpha = .001$.	Rejected
$p < .001$	***	The test was significant at all levels	Rejected

Hypothesis testing:
two samples

10-2: Inference for a Difference in Means of Two Normal Distributions, Variances Known

Figure 10-1 Two independent populations.

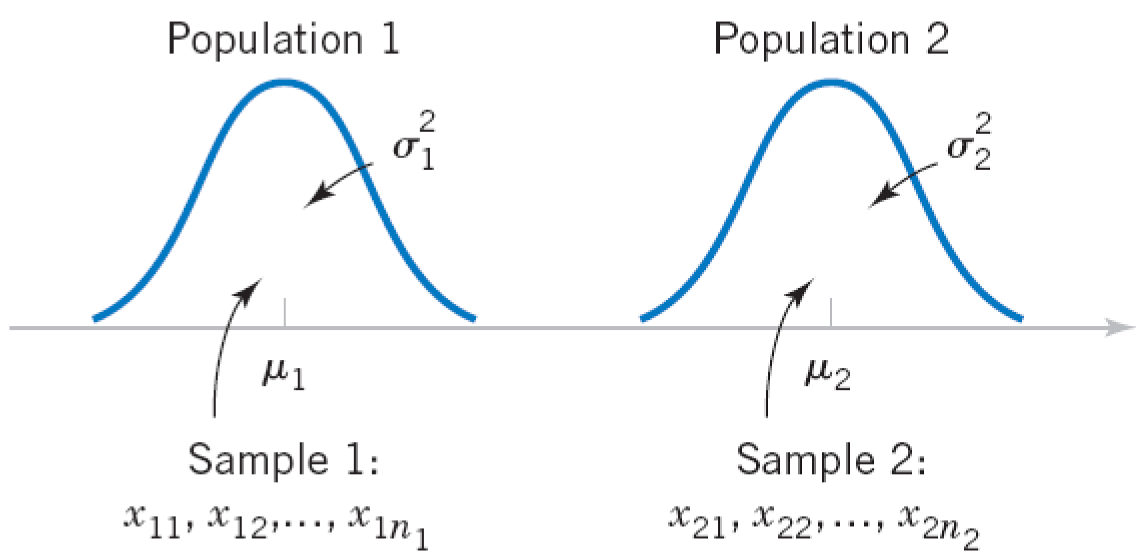


Figure 10-1 Two independent populations.

10-2: Inference for a Difference in Means of Two Normal Distributions, Variances Known

Assumptions

1. $X_{11}, X_{12}, \ldots, X_{1n_1}$ is a random sample from population 1.
2. $X_{21}, X_{22}, \ldots, X_{2n_2}$ is a random sample from population 2.
3. The two populations represented by X_1 and X_2 are independent.
4. Both populations are normal.

$$E(\overline{X}_1 - \overline{X}_2) = E(\overline{X}_1) - E(\overline{X}_2) = \mu_1 - \mu_2$$

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

10-2: Inference for a Difference in Means of Two Normal Distributions, Variances Known

The quantity

$$Z = \frac{\overline{X}_1 - \overline{X}_2 - (\mu_1 - \mu_2)}{\sqrt{\dfrac{\sigma_1^2}{n_1} + \dfrac{\sigma_2^2}{n_2}}} \qquad (10\text{-}1)$$

has a $N(0, 1)$ distribution.

10-2: Inference for a Difference in Means of Two Normal Distributions, Variances Known

10-2.1 Hypothesis Tests for a Difference in Means, Variances Known

Null hypothesis: $H_0\colon \mu_1 - \mu_2 = \Delta_0$

Test statistic:

$$Z_0 = \frac{\overline{X}_1 - \overline{X}_2 - \Delta_0}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}} \tag{10-2}$$

Alternative Hypotheses	***P*-Value**	**Rejection Criterion For for Fixed-Level Tests**
$H_1\colon \mu_1 - \mu_2 \neq \Delta_0$	Probability above $\lvert z_0\rvert$ and probability below $-\lvert z_0\rvert$, $P = 2[1 - \Phi(\lvert z_0\rvert)]$	$z_0 > z_{\alpha/2}$ or $z_0 < -z_{\alpha/2}$
$H_1\colon \mu_1 - \mu_2 > \Delta_0$	Probability above z_0, $P = 1 - \Phi(z_0)$	$z_0 > z_\alpha$
$H_1\colon \mu_1 - \mu_2 < \Delta_0$	Probability below z_0, $P = \Phi(z_0)$	$z_0 < -z_\alpha$

10-2.1 Hypotheses Tests on the Difference in Means, Variances Unknown

Case 2: $\sigma_1^2 \neq \sigma_2^2$

If H_0: $\mu_1 - \mu_2 = \Delta_0$ is true, the statistic

$$T_0^* = \frac{\overline{X}_1 - \overline{X}_2 - \Delta_0}{\sqrt{\dfrac{S_1^2}{n_1} + \dfrac{S_2^2}{n_2}}} \qquad (10\text{-}15)$$

is distributed as t-distribution with degrees of freedom given by

$$v = n_1 + n_2 - 2,$$

or more generally

Let's do a sweet exercise #1

Example 10-7 **Chocolate and Cardiovascular Health** An article in *Nature* (2003, Vol. ~~48,~~ 424 p. 1013) described an

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Plasma antioxidants from chocolate

Dark chocolate may offer its consumers health benefits the milk variety cannot match.

There is some speculation that dietary flavonoids from chocolate, in particular (−)epicatechin, may promote cardiovascular health as a result of direct antioxidant effects or through antithrombotic mechanisms[1–3]. Here we show that consumption of plain, dark chocolate (Fig. 1) results in an increase in both the total antioxidant capacity and the (−)epicatechin content of blood plasma, but that these effects are markedly reduced when the chocolate is consumed with milk or if milk is incorporated as milk chocolate. Our findings indicate that milk may interfere with the absorption of antioxidants from chocolate *in vivo* and may therefore negate the potential health benefits that can be derived from eating moderate amounts of dark chocolate.

To determine the antioxidant content of different chocolate varieties, we took dark chocolate and milk chocolate prepared from the same batch of cocoa beans and defatted them twice with *n*-hexane before extracting them with a mixture of water, acetone and acetic acid (70.0:29.8:0.2 by volume). We measured their *in vitro* total antioxidant capacities using the ferric-reducing antioxidant potential (FRAP) assay[4]; FRAP reduced iron per 100 g for dark and milk chocolate, respectively. Volunteers must therefore consume twice as much milk chocolate as dark chocolate to receive a similar intake of antioxidants.

We recruited 12 healthy volunteers (7 women and 5 men with an average age of 32.2 ± 1.0 years (range, 25–35 years). Subjects were non-smokers, had normal blood lipid levels, were taking no drugs or vitamin supplements, and had an average weight of 65.8 ± 3.1 kg (range, 46.0–86.0 kg) and body-mass index of 21.9 ± 0.4 kg m^{-2} (range, 18.6–23.6 kg m^{-2}). On different days, following a crossover experimental design, subjects consumed 100 g dark chocolate, 100 g dark chocolate with 200 ml full-fat milk, or 200 g milk chocolate (containing the equivalent of up to 40 ml milk).

One hour after subjects had ingested the chocolate, or chocolate and milk, we measured the total antioxidant capacity of their plasma by FRAP assay. Plasma antioxidant levels increased significantly after consumption of dark chocolate alone, from $100 \pm 3.5\%$ to $118.4 \pm 3.5\%$ (*t*-test, $P < 0.001$), returning to baseline values ($95.4 \pm 3.6\%$) after 4 h (Fig. 2a). There was


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Mauro Serafini*, Rossana Bugianesi*, Giuseppe Maiani*, Silvia Valtuena*, Simone De Santis*, Alan Crozier†

**Antioxidant Research Laboratory, Unit of Human Nutrition, National Institute for Food and Nutrition Research, Via Ardeatina 546, 00178 Rome, Italy*
e-mail: serafini@inran.it
†*Plant Products and Human Nutrition Group, Graham Kerr Building, Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK*

Figure 1 Stack of benefits? Unlike its milky counterpart, dark chocolate may provide more than just a treat for the tastebuds.

could be due to the formation of secondary bonds between chocolate flavonoids and milk proteins[6,7], which would reduce the biological accessibility of the flavonoids and therefore the chocolate's potential antioxidant properties *in vivo*.

Our findings highlight the possibility

Vol. 424

Sweet matlab exercise #1

- **dark=[118.8 122.6 115.6 113.6 119.5 115.9 115.8 115.1 116.9 115.4 115.6 107.9];**
- **milk=[102.1 105.8 99.6 102.7 98.8 100.9 102.8 98.7 94.7 97.8 99.7 98.6]**
- **You can download it at the course website**
- **Calculate P-value of the null hypothesis H_0 that milk = dark against H_1 that dark > milk**

Sweet matlab exercise #1

- **dark=[118.8 122.6 115.6 113.6 119.5 115.9 115.8 115.1 116.9 115.4 115.6 107.9];**
- **milk=[102.1 105.8 99.6 102.7 98.8 100.9 102.8 98.7 94.7 97.8 99.7 98.6]**
- **x_dark=mean(dark) % sample mean dark chocolate**
- **x_milk=mean(milk) % sample mean milk chocolate**
- **s_dark=std(dark) % sample std dark chocolate**
- **s_milk=std(milk) % sample std milk chocolate**
- **n=12 % % sample size of both dark and milk**
- **std_xdiff=sqrt(s_dark.^2./2+s_milk.^2./n) % std diff x**
- **z_stat=(x_dark-x_milk)./std_xdiff % z-statistic**
- **P_value_z=erfc (z_stat./sqrt(2))./2 %P-value null true**

Credit: XKCD comics