Faculty of Health Sciences

Day 7: Multiple linear regression, confounding, interaction

Paul Blanche

Section of Biostatistics, University of Copenhagen

May 10, 2023

Outline

The multiple linear model

- ILO: to outline what the multiple linear model is about
- ILO: to list important methods that are special cases of the model
- ILO: to describe the connection with the t-test

Why multiple regression?

- ILO: to exemplify when a multiple regression can be better than a univariate analysis
- ILO: to describe the connection with ANOVA

ANCOVA and model checking

- ILO: to use the ANCOVA and interpret the results
- ILO: to evaluate some modeling assumption

Digression: Table-I and the statistical analysis plan (SAP)

- ILO: to repeat widely recommended practices in statistics

Interaction and subgroup analysis

- ILO: to interpret models with interaction and exemplify their usefulness
- ILO: to contrast the use of these models and subgroup analyses

Case: vitamin D data

Data, n=412:

```
country vitd    age    bmi vitdintake
      1 22.4 11.888 19.254      7.188
      1 37.0 12.441 17.567      1.186
      1 12.9 13.025 17.700      1.480
      1 13.6 13.501 16.953      1.612
      1  9.1 12.474 20.806      3.940
      1 13.4 12.973 18.242      8.152
```

(also data on sun exposure: sunexp)

Outcome: vitamin D measured in morning blood samples, after an overnight fast (nmol/l).

Reference:

- Andersen and Skovgaard. *Regression with linear predictors*. Springer, 2010.
- Andersen et al., Eur. J. Clin. Nutr. (2005)

Note: the slides of today borrow many examples and explanations presented in more details in the above textbook reference.

Remarks on the case study and log-transformation

- It is common, and often sensible, to study the log of a concentration, instead of the concentration itself, when using linear regression. This is because:
  - concentration cannot be negative.
  - the variability between observations is often higher for higher concentrations.
- We will log-transform in our case study:

$$\text{outcome} = \log_{10}(\text{vitamin D concentration}) \quad .$$

[1] See e.g. Keene "The log transformation is special." Statistics in Medicine 14.8 (1995): 811-819.

Remarks on the case study and log-transformation

- It is common, and often sensible, to study the log of a concentration, instead of the concentration itself, when using linear regression. This is because:
  - concentration cannot be negative.
  - the variability between observations is often higher for higher concentrations.
- We will log-transform in our case study:

$$\text{outcome} = \log_{10}(\text{vitamin D concentration}) \quad .$$

- But, it is not always needed and important to log-transform!

DO NOT SYSTEMATICALLY LOG-TRANSFORM WITHOUT A GOOD REASON!

- It is best to pre-specify the choice of log-transforming or not based on background knowledge (i.e. your experience of that of others reported in the literature).[1]
- Sometimes, but not always, it is interesting to present and interpret the results on the original scale, using the back-transformation ($\exp$).

[1] See e.g. Keene "The log transformation is special." Statistics in Medicine 14.8 (1995): 811-819.

The multiple linear model

The i-th observation (e.g. from subject i) of the outcome Y is described as:

$$Y_i = \alpha + \beta_1 x_i + \beta_2 z_i + \cdots + \varepsilon_i$$

- x_i, z_i, ... are the predictor (i.e. explanatory) variables / covariates.
- the linear predictor $\alpha + \beta_1 x_i + \beta_2 z_i + \ldots$ is the mean outcome for any subject i having covariate values x_i, z_i, ...
- ε_i's are individual 'error' terms ("random/unexplained deviation from the mean") assumed normally distributed with zero mean and the **same** variance σ^2_ε regardless of the values x_i, z_i, ...

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

1. Individual observations are independent.
2. The variance of 'error' terms is the same for all groups (homogeneity).
3. 'Error' terms are normally distributed.

The multiple model generalizes simpler models

Many simple settings can be thought as a special case of the multiple linear model.

Which and why?

- t-test (Lecture 2)
  - one binary predictor variable
- univariate linear model (Lecture 3)
  - one quantitative predictor variable
- ANOVA (Lecture 4)
  - one categorical predictor variable (one-way ANOVA)
  - two categorical predictor variables (two-way ANOVA)
- ANCOVA (today's Lecture)
  - one categorical and one quantitative predictor variable

Note: this holds when using t-test and ANOVA that assume the same standard deviation for all groups (which is not the default/recommended choice for the t-test)

Case: one binary variable

- Research question: is the mean log vitamin D different between elderly women ($>$ 69) having a "normal" weight and those being "overweight"?
- Predictor variable(s): body mass index "normal" (18.5-25) or "overweight" ($>$25).
- Data example: Irish women, $n = 42$ $(16 + 25)$.
- Linear model:

$$Y_i = \alpha + \beta z_i + \varepsilon_i$$

with

$$z_i = \begin{cases} 1 & \text{if } i \text{ is "overweight"} \\ 0 & \text{if } i \text{ has a "normal" weight} \end{cases}$$

  - α: mean for "normal" weight
  - $\alpha + \beta$: mean for "overweight"
  - β: difference in mean between "overweight" and "normal"

R code & default output

R code:

```
vitaminD$bmigroup <- factor(as.numeric(vitaminD$bmi > 25))
lm1 <- lm(log10(vitd)~bmigroup,data=irlwomen)
summary(lm1)
```

Output:

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  1.71987    0.04554  37.765   <2e-16 ***
bmigroup1   -0.12682    0.05832  -2.175   0.0358 *
```

R code & default output

R code:

```
vitaminD$bmigroup <- factor(as.numeric(vitaminD$bmi > 25))
lm1 <- lm(log10(vitd)~bmigroup,data=irlwomen)
summary(lm1)
```

Output:

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  1.71987    0.04554  37.765   <2e-16 ***
bmigroup1   -0.12682    0.05832  -2.175   0.0358 *
```

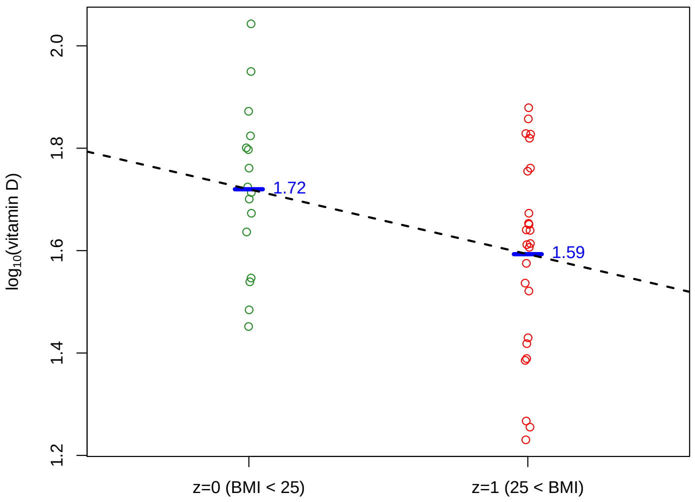
R code:

```
tapply(log10(irlwomen$vitd), irlwomen$bmigroup, mean)
diff(tapply(log10(irlwomen$vitd), irlwomen$bmigroup, mean))
```

Output:

```
1.719873 1.593053
-0.1268206
```

Visualizing the raw data & results



The regression line passes through the sample means, i.e. the two estimated means corresponds to the sample means in each group.

Formatted results and comparison to that of `t.test()`

R code:

```
FormatResLm <- function(fit){
 cbind.data.frame(round(cbind(Est=fit$coef,confint(fit)),2),
                'p-value'=format.pval(summary(fit)$coefficients[,"Pr(>|t|)"],
                                    digits=3))}
FormatResLm(lm1)
```

Output:

```
              Est 2.5 % 97.5 % p-value
(Intercept)  1.72  1.63   1.81  <2e-16
bmigroup1   -0.13 -0.24  -0.01  0.0358
```

R code:

```
t.test(log10(irlwomen$vitd) ~ irlwomen$bmigroup,var.equal=TRUE)
```

Output:

```
Two Sample t-test

data:  log10(irlwomen$vitd) by irlwomen$bmigroup
t = 2.1745, df = 39, p-value = 0.0358
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 0.008853898 0.244787253
sample estimates:
mean in group 0 mean in group 1
       1.719873        1.593053
```

Conclusions with only one binary variable

- Model estimates match the observed means in each group.
- The estimated regression coefficient (slope) is identical to the difference between the sample means.
- The p-values computed by the linear model and the t-test, when assuming equal variances in the two groups, are identical.
- The confidence interval for the regression coefficient (slope) is identical to that computed along the t-test to complement the p-value, when assuming equal variances in the two groups are identical.

Furthermore: similar remarks about identical results for the **ANOVA** case. That is why we were already using the `lm()` function of R in the ANOVA case (although R and other software have also specific function for ANOVA analyses).

Digression: median and back-transformation (1/2)

R code:

```
rbind(Mean=tapply(X=log10(irlwomen$vitd), INDEX=irlwomen$bmigroup, FUN=mean),
      Median=tapply(X=log10(irlwomen$vitd), INDEX=irlwomen$bmigroup, FUN=median))
```

Output:

```
              0        1
Mean   1.719873 1.593053
Median 1.718883 1.613842
```

Here, because the "model" for the mean is a good model for the median (M), because median($\log(Y)$)=$\log$(median(Y)) and:

$$\log_{10}(\widehat{M}_1) - \log_{10}(\widehat{M}_0) = \log_{10}\left(\frac{\widehat{M}_1}{\widehat{M}_0}\right) = -0.12682$$

then $\widehat{M}_1/\widehat{M}_0 = 10^{-0.12682} = 0.75$; hence we can conclude that we estimate that overweight women have a 25% lower median vitamin D concentration compared to the normal weight women.[2]

[2] See similar remarks in Lecture 3.

Digression: median and back-transformation (2/2)

We do not model the means in each group on the original scale via the parameters α and β only . Only the median in each group on the original scale depend on parameters α and β only. Unlike the medians, the means also depend on σ_ε. However, the ratio of the means is modeled solely via β, as 10^β (because we used $\log_{10}$ here[3]).

Hence, we can also conclude that we estimate that overweight women have a 25% lower mean vitamin D concentration than that of normal weight women.

Take-home message: using linear regression we always model means and difference of means. But, when used together with a log-transformation, linear regression additionally models ratios of means on the original scale.

Details: this is because according to our model and the mathematical properties of the log-normal distribution, we model the two mean vitamin D concentrations in each group as $10^{\alpha+\sigma_\varepsilon^2/2}$ and $10^{\alpha+\beta+\sigma_\varepsilon^2/2}$.

[3] if we had used $\log_2$, it would have been 2^β; if we had used the "natural" $\log$, it would have been e^β, ...

Outline

The multiple linear model

ILO: to outline what the multiple linear model is about
ILO: to list important methods that are special cases of the model
ILO: to describe the connection with the t-test

Why multiple regression?

ILO: to exemplify when a multiple regression can be better than a univariate analysis
ILO: to describe the connection with ANOVA

ANCOVA and model checking

ILO: to use the ANCOVA and interpret the results
ILO: to evaluate some modeling assumption

Digression: Table-I and the statistical analysis plan (SAP)

ILO: to repeat widely recommended practices in statistics

Interaction and subgroup analysis

ILO: to interpret models with interaction and exemplify their usefulness
ILO: to contrast the use of these models and subgroup analyses

Why multiple regression?

- better adjusting / explaining (main focus in this course)
- better predict or gain power (more advanced topic, touched upon in lecture 4)

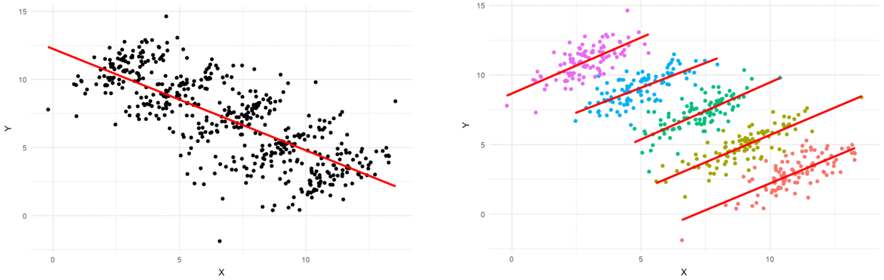
Same reasons as for why logistic regression can be more useful than simpler 2x2 tables analyses.

- Useful when we want to make comparisons with respect to one factor/variable (e.g. treatment or exposure) among individuals otherwise similar with respect to other variables that we adjust for (e.g. age, sex, comorbidity...).
- Multiple regression is a tool to deal with confounding and unbalanced designs.
- Multiple regression offers an alternative to stratification (i.e. subgroup analysis) when the data are not very large or/and we can assume that some differences are “similar” within different subgroups (→).

Multiple regression to limit confounding

We often compare two groups with the aim to get a “tentative” causal interpretation of the statistical association that we can show. To do so, we adjust on some variables to make a comparison among subjects as similar as possible with respect to some relevant variables.

Extreme hypothetical example of confounding:



Case: comparing countries

Step 1: Initial research question: *“Is the average log-vitamin D different in the Irish and Polish population of elderly women?”*

Step 2: Quick look at the collected data via a typical “Table I”:

		Ireland (n=41)	Poland (n=65)
Age	median [iqr]	72[70.8, 73.3]	71.7[70.4, 72.6]
BMI	18.5-25	16(39%)	12(19%)
	> 25	25(61%)	53(81%)
Sun exposure	avoid	16(39%)	26(40%)
	sometimes	21(51%)	34(52%)
	prefer	4(10%)	5(8%)
Vitamin D intake	median [iqr]	5.5[3.2, 12.1]	5.2[3.0, 11.9]

Step 3: Updated research question: *“Is there a difference in average log-vitamin D between Irish and Polish elderly women having the same BMI group?”*[4]

[4] More about the rational for this “3 steps approach” in the next section.

Case: comparing countries while “adjusting” for BMI group

- Research question: is there a difference in average log-vitamin D between Irish and Polish elderly women having the same BMI group?
- Predictor variable(s):
  - BMI “normal” (18.5-25) / “overweight” (>25).
  - Country Ireland / Poland
- Data example: Irish and Poland women, $n = 106\ (41 + 65)$.
- Linear model: $Y_i = \alpha + \beta_1 x_i + \beta_2 z_i + \varepsilon_i$

$$x_i = \begin{cases} 1 & \text{if } i \text{ is Polish} \\ 0 & \text{if } i \text{ is Irish} \end{cases} \qquad z_i = \begin{cases} 1 & \text{if } i \text{ is "overweight"} \\ 0 & \text{if } i \text{ has a "normal" weight} \end{cases}$$

This is a two-way ANOVA model! (without interaction)

Parameters interpretation

According to the model, the means of log-vitamin D are:

Country / BMI	Ireland	Poland
"Normal"	α	$\alpha + \beta_1$
"Overweight"	$\alpha + \beta_2$	$\alpha + \beta_1 + \beta_2$

- α: mean outcome for Irish with "normal" BMI (reference group).
- β_1: difference in mean outcome between Irish and Polish among women of the same BMI group (whatever it is).
- β_2: difference in mean outcome between women with "overweight" and those having a "normal" BMI, among women of the same country (whatever it is).

R code & default output

R code:

```
lm2 <- lm(log10(vitd) ~  Country + bmigroup, data = irlpolwomen)
summary(lm2)
```

Output:

```
Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    1.72854    0.04016  43.040  < 2e-16 ***
CountryPoland -0.14164    0.03947  -3.589 0.000511 ***
bmigroup1     -0.14103    0.04360  -3.235 0.001638 **
```

Conclusions?

Results with 95% CIs

R code:

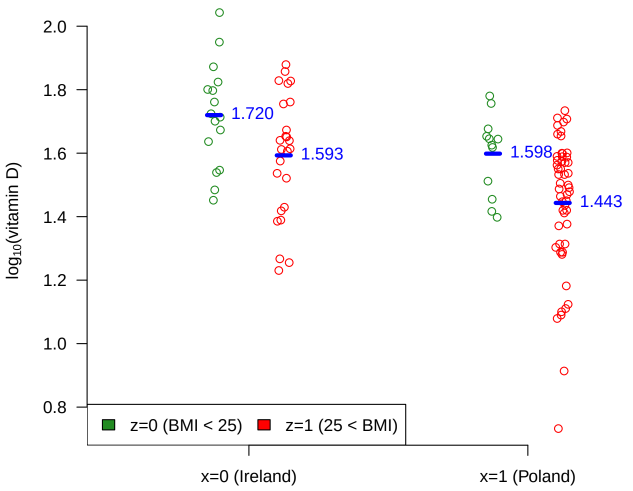
```
FormatResLm(lm2)
```

Output:

```
                Est 2.5 % 97.5 %  p-value
(Intercept)    1.73  1.65   1.81  < 2e-16
CountryPoland -0.14 -0.22  -0.06 0.000511
bmigroup1     -0.14 -0.23  -0.05 0.001638
```

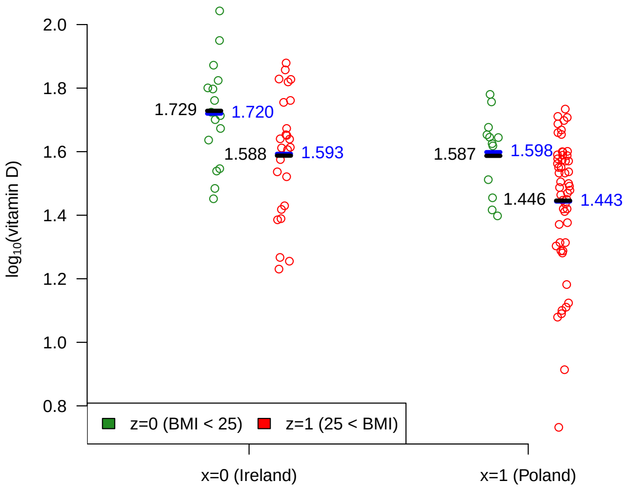
Conclusions?

Visualizing the raw data



- Observed (sample) means: blue

Visualizing the raw data & results



- Observed (sample) means: blue
- Estimated means (from the model): black

Outline

The multiple linear model
ILO: to outline what the multiple linear model is about
ILO: to list important methods that are special cases of the model
ILO: to describe the connection with the t-test

Why multiple regression?
ILO: to exemplify when a multiple regression can be better than a univariate analysis
ILO: to describe the connection with ANOVA

ANCOVA and model checking
ILO: to use the ANCOVA and interpret the results
ILO: to evaluate some modeling assumption

Digression: Table-I and the statistical analysis plan (SAP)
ILO: to repeat widely recommended practices in statistics

Interaction and subgroup analysis
ILO: to interpret models with interaction and exemplify their usefulness
ILO: to contrast the use of these models and subgroup analyses

Case: comparing countries while "adjusting" for BMI

- Research question: is there a difference in mean log-vitamin D between Irish and Polish elderly women having the same BMI?
- Predictor variable(s):
  - BMI as a quantitative (continuous) variable
  - Country Ireland / Poland
- Data example: Irish and Poland women, $n = 106\ (41 + 65)$.
- Linear model: $Y_i = \alpha + \beta_1 x_i + \beta_2 z_i + \varepsilon_i$

$$x_i = \begin{cases} 1 & \text{if } i \text{ is Polish} \\ 0 & \text{if } i \text{ is Irish} \end{cases} \qquad z_i = \text{BMI of subject } i.$$

This is a called an **ANCOVA** model (ANalysis of COVAriance), because we "adjust" with a quantitative/continuous covariate.

- α: mean outcome for Irish (x=0) with BMI=0 (z=0) (meaningless!)
- β_1: difference in mean outcome between Polish and Irish among women having the same BMI (whatever it is).
- β_2: difference in mean outcome between two women, one having a BMI one unit higher than the other ($z + 1$ versus z), among women of the same country (whatever it is).

  Note: this holds whatever the two BMI values being compared, as long as there is a one unit difference between the two. This is the so called "linearity assumption".

R code:

```
irlpolwomen$bmi5 <- irlpolwomen$bmi/5
lm3 <- lm(log10(vitd) ~ bmi5 + Country, data = irlpolwomen)
summary(lm3)
```

Output:

```
Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    2.04273    0.12291  16.620  < 2e-16 ***
bmi5          -0.07593    0.02262  -3.357  0.00110 **
CountryPoland -0.13135    0.04005  -3.280  0.00142 **
```

R code:

```
irlpolwomen$bmi5 <- irlpolwomen$bmi/5
lm3 <- lm(log10(vitd) ~ bmi5 + Country, data = irlpolwomen)
summary(lm3)
```

Output:

```
Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    2.04273    0.12291  16.620  < 2e-16 ***
bmi5          -0.07593    0.02262  -3.357  0.00110 **
CountryPoland -0.13135    0.04005  -3.280  0.00142 **
```

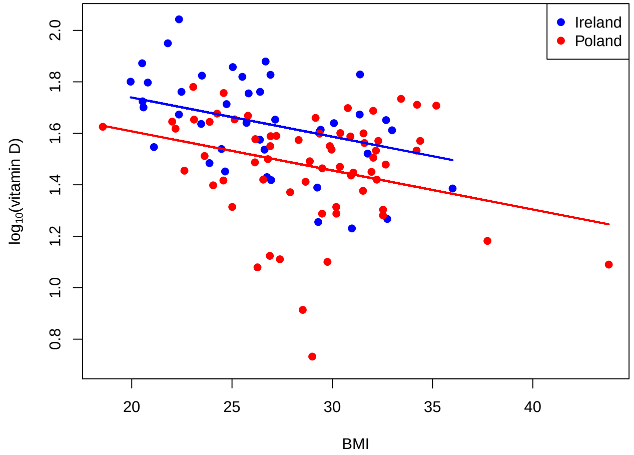
R code:

```
FormatResLm(lm3)
```

Output:

```
                Est 2.5 % 97.5 % p-value
(Intercept)    2.04  1.80   2.29 < 2e-16
bmi5          -0.08 -0.12  -0.03 0.00110
CountryPoland -0.13 -0.21  -0.05 0.00142
```

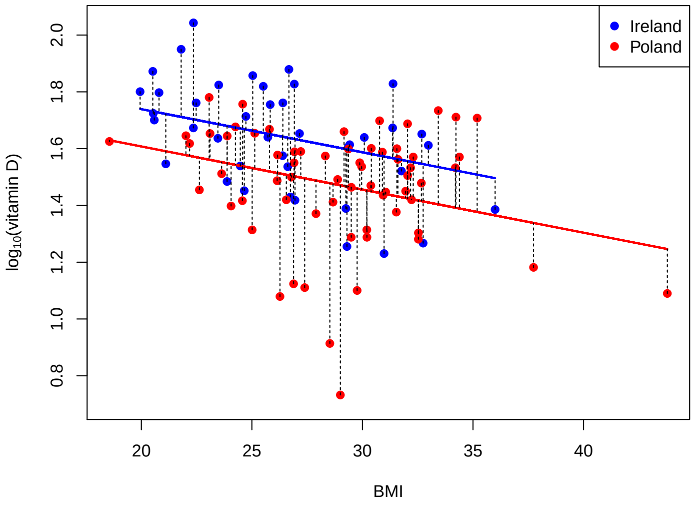
Visualizing the raw data & results



Note: an ANCOVA model is simply a regression model for parallel regression lines.

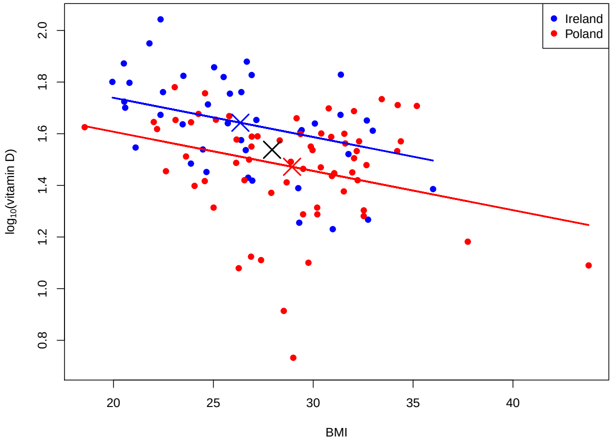
- β_2: is the common slope of the two lines.
- β_1: is the size of the vertical distance between the two lines.

Estimate of σ_ε (standard deviation of the 'error' terms)



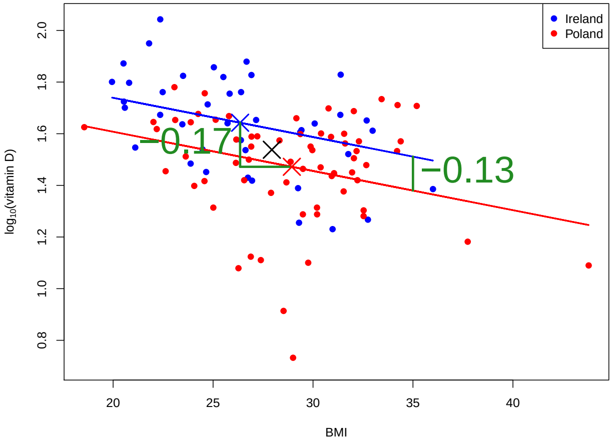
In the output of the summary(), "Residual standard error: 0.1921" is the estimate of σ_ε. It is computed "nearly" as the standard deviation of the residuals represented by the horizontal black dashed bars. It quantifies the vertical "spread" o the individual observations below/above the corresponding regression lines.

Comparing adjusted and unadjusted results



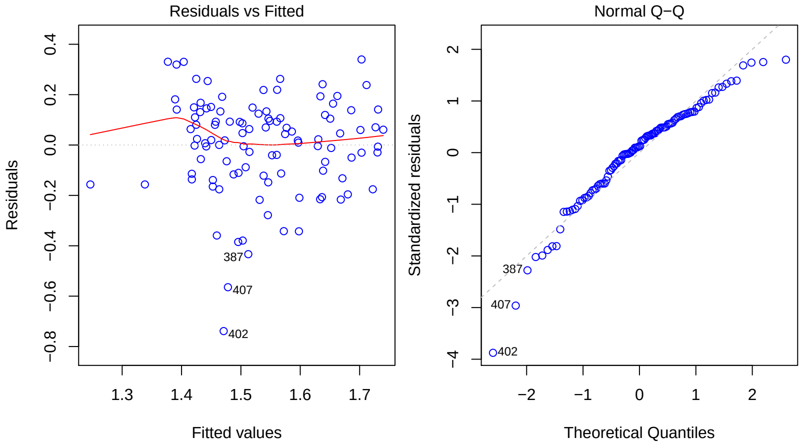
- crosses represent the mean of BMI (x-axis) and outcome (y-axis) of the entire sample (black) and for each country.

Comparing adjusted and unadjusted results



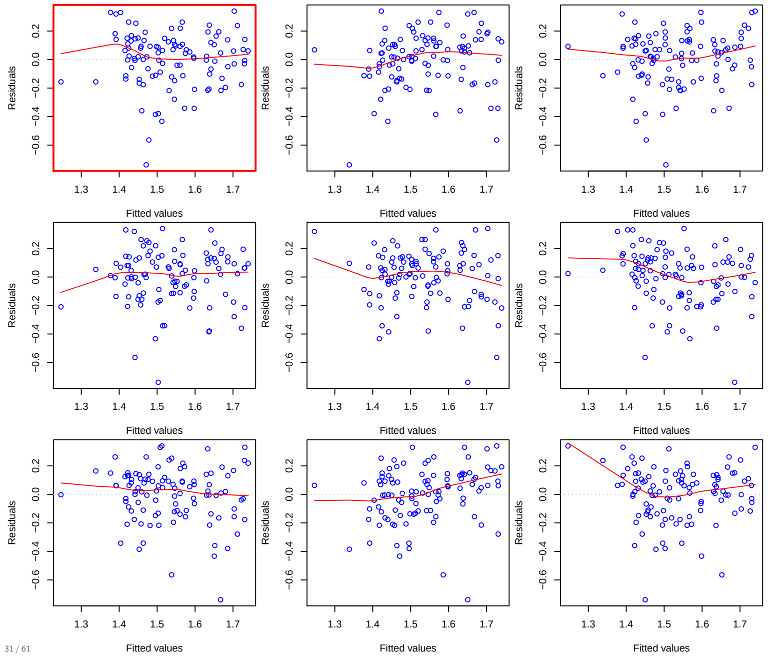
- crosses represent the mean of BMI (x-axis) and outcome (y-axis) of the entire sample (black) and for each country.
- Because the mean BMI is not the same in the two countries and because BMI is associated to the level of vitamin D, the adjusted and non-adjusted results are different.
- unadjusted difference between countries is -0.17, adjusted is -0.13.

Model checking (default) plots

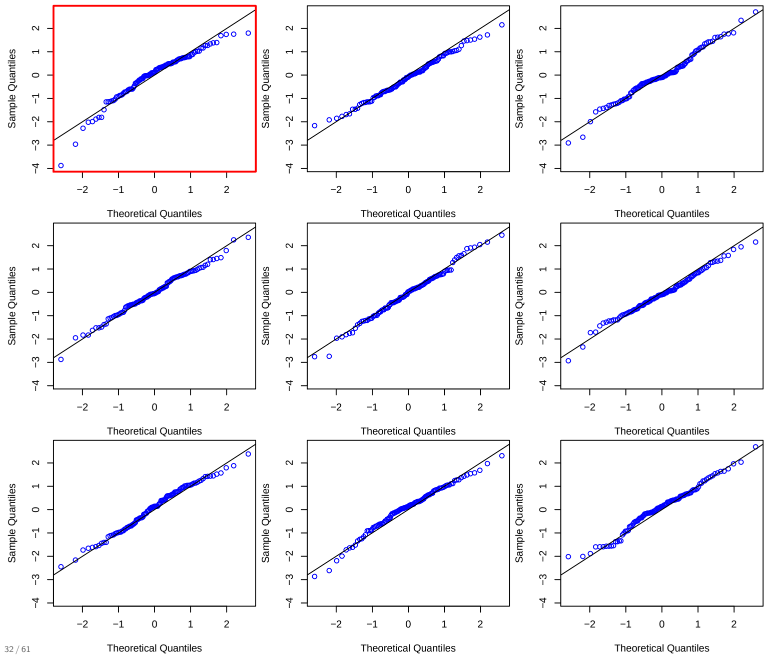


- Residual plot: always "important".
- QQplot: mostly for small samples and when computing prediction intervals.
- Similar importance, for similar reasons, as in univariate linear regression (Lecture 3) and ANOVA model (Lecture 4).

Wally residual plot



Wally QQplot



ANCOVA with more than two categories

- Research question: is there a difference in mean log-vitamin D between **Danish, Finnish, Irish and Polish** elderly women having the same BMI?
- Predictor variable(s):
  - BMI as a quantitative (continuous) variable
  - Country Denmark / Finland / Ireland / Poland
- Data example: all elderly women, $n = 213\ (53 + 54 + 41 + 65)$.
- Linear model: $Y_i = \alpha + \beta_1 x_i + \beta_2 z_i + \beta_3 v_i + \beta_4 w_i + \varepsilon_i$

$$x_i = \text{BMI of subject } i \qquad z_i = \begin{cases} 1 & \text{if } i \text{ is Finnish} \\ 0 & \text{otherwise} \end{cases}$$

$$v_i = \begin{cases} 1 & \text{if } i \text{ is Irish} \\ 0 & \text{otherwise} \end{cases} \qquad w_i = \begin{cases} 1 & \text{if } i \text{ is Polish} \\ 0 & \text{otherwise} \end{cases}$$

Note: $z_i = v_i = w_i = 0$ for Danish women (reference group).

- β_2: difference in mean outcome between Finnish and Danish among women having the same BMI (whatever it is).
- β_3 & β_4: same but between Irish and Danish & Polish and Danish.

- β_2: difference in mean outcome between Finnish and Danish among women having the same BMI (whatever it is).
- β_3 & β_4: same but between Irish and Danish & Polish and Danish.

As in the simpler ANOVA context, we can test the global null hypothesis

"H_0: *there is no difference in mean log-vitamin level between women of the four countries, when comparing women of the same BMI*",

that is

$$\boxed{H_0 : \beta_2 = \beta_3 = \beta_4 = 0}$$

Similarly as in the (simpler) ANOVA context, we can use either:

- F-test
- min-P method

Pros and cons are similar to those in the ANOVA context (Lecture 4).

R code & default output

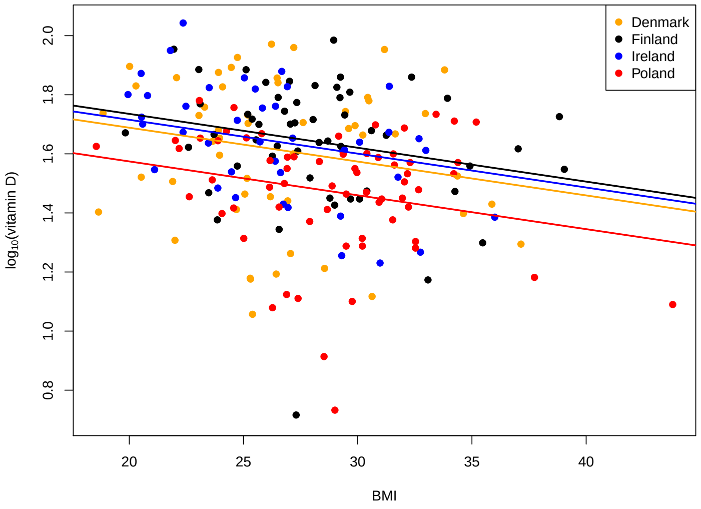
R code:

```
lm4 <- lm(log10(vitd) ~ bmi5 + Country, data = dwomen)
summary(lm4)
```

Output:

```
Coefficients:
               Estimate Std. Error t value Pr(>|t|)
(Intercept)     1.91780    0.09710  19.750  < 2e-16 ***
bmi5           -0.05732    0.01746  -3.282  0.00121 **
CountryFinland  0.04674    0.04128   1.132  0.25891
CountryIreland  0.02683    0.04390   0.611  0.54170
CountryPoland  -0.11415    0.03995  -2.857  0.00471 **
```

Visualizing the raw data & results



R code:

```
anova(lm(log10(vitd) ~ bmi5, data = dwomen),
      lm(log10(vitd) ~ bmi5 + Country, data = dwomen))
```

Output:

```
Analysis of Variance Table

Model 1: log10(vitd) ~ bmi5
Model 2: log10(vitd) ~ bmi5 + Country
  Res.Df     RSS Df Sum of Sq      F    Pr(>F)
1    211 10.1690
2    208  9.2635  3    0.9055 6.7773 0.0002212 ***
```

Comments:

- F-test p-value=0.0002212 is significant: there is a difference between countries, for the average outcome, when comparing women of the same BMI. But which differences?
- To avoid coding mistakes and misunderstings of R output do compare the two models: do not use "anova(lm4)".

Recommended analysis (see R-demo for code)

Statistical methods:
Comparisons between countries were made with a multiple linear model to adjust on BMI (ANCOVA). P-values and 95% confidence intervals were adjusted for multiple testing using the min-P method (aka max-t test) as implemented in the multcomp-package [ref.[5]] of the statistical software R [ref.[6]] and described in [ref.[7]].

Results (adjusted for multiple testing):

Comparison	Est. Diff	95% CI	p-value
Finland - Denmark	0.05	[-0.06; 0.15]	0.6695
Ireland - Denmark	0.03	[-0.09; 0.14]	0.9282
Poland - Denmark	-0.11	[-0.22;-0.01]	0.0239
Ireland - Finland	-0.02	[-0.13; 0.09]	0.9695
Poland - Finland	-0.16	[-0.26;-0.06]	0.0003
Poland - Ireland	-0.14	[-0.25;-0.03]	0.0069

Note:

- Significant association between countries and log vitamin D after adjusting on BMI, p-value= 0.0003 (i.e. the minimum). And we also know where the differences are!
- Similarly, we can use the method for the **"many-to-one"** setting (as in Lecture 4).
- This method works with any linear model, not just an ANCOVA (and so does the F-test).

[5] Hothorn, Bretz & Westfall (2008). Simultaneous Inference in General Parametric Models. Biometrical Journal 50(3), 346–363.

[6] R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

[7] Bretz, Hothorn, & Westfall (2016). Multiple comparisons using R. CRC Press.

Outline

The multiple linear model

- ILO: to outline what the multiple linear model is about
- ILO: to list important methods that are special cases of the model
- ILO: to describe the connection with the t-test

Why multiple regression?

- ILO: to exemplify when a multiple regression can be better than a univariate analysis
- ILO: to describe the connection with ANOVA

ANCOVA and model checking

- ILO: to use the ANCOVA and interpret the results
- ILO: to evaluate some modeling assumption

Digression: Table-I and the statistical analysis plan (SAP)

- ILO: to repeat widely recommended practices in statistics

Interaction and subgroup analysis

- ILO: to interpret models with interaction and exemplify their usefulness
- ILO: to contrast the use of these models and subgroup analyses

Digression: How to adjust?

There is usually no unique "best" way to choose the variables to adjust on, but several interesting options, all with pros and cons. But, the choice should be supported by:

- Research question
  - Which groups do we want to compare? In which population? Among subjects similar with respect to what?
- Background knowledge
  - Why these groups? Why these population and comparisons among these "similar" subjects?
- Available data (variables & sample size)
  - What is the best compromise between what we ideally want to do and what we can do?

Note: several models may be needed when there are several research questions.[8]

[8] See e.g. Westreich & Greenland (2013). The table 2 fallacy: presenting and interpreting confounder and modifier coefficients. American journal of epidemiology, 177(4), 292-298.

Digression: Table 1

- Using a simple descriptive "Table 1" to informally compare the distribution of all variables (except the outcome) between the groups we want to compare often helps to choose how we should adjust.
- It is often useful to adjust on age, gender, baseline comorbidities etc or any variable which is not equally distributed between the groups[9].
- This is fine and not "cheating" (i.e. not "data snooping" or "p-hacking") as long we do not look at any association between the outcome and any variable before we make the choice on how to adjust. Of course, full pre-specification is even better, if possible[10].
- The aim of this descriptive "Table 1" is often only to describe the population of each group, hence it usually makes sense and is recommended that it does not include p-values.[11]

[9] and which is not a consequence of the treatments or exposures being studied

[10] e.g. for clinical trials, often we can and we should fully prespecify.

[11] See e.g. STROBE or CONSORT statements endorsed by most medical journals.

Digression: efficient "Table 1" making in R ("Publish" R package)

R code:

```
Tab1ex <- univariateTable(Country~age+Q(bmi)+Q(vitdintake)+sunexp,
                          data=IrFinPo,
                          compare.groups = FALSE,
                          show.totals = FALSE)
Tab1ex
```

Output:

```
   Variable        Level    Denmark (n=78)    Finland (n=88)    Ireland (n=54)
        age    mean (sd)       52.7 (27.7)         49.1 (29)       57.8 (25.9)
        bmi median [iqr] 24.9 [20.9, 27.5] 25.5 [20.8, 28.8] 24.9 [22.4, 28.9]
 vitdintake median [iqr]  6.1 [ 2.7, 11.6]  7.9 [ 5.0, 15.2]  5.3 [ 2.9, 10.5]
     sunexp        avoid         14 (17.9)         15 (17.0)         18 (33.3)
               sometimes         43 (55.1)         42 (47.7)         25 (46.3)
                  prefer         21 (26.9)         31 (35.2)         11 (20.4)
```

Notes: remember, a "common task" is rarely time-consuming to R users. Packages and specific functions are continuously created to help with common needs. Spend a few minutes to search f appropriate packages!

Digression: statistical analysis plan (SAP)

- It is strongly recommended to make a statistical analysis plan (SAP) before starting any analysis on the outcome data. This is a must for confirmatory research (e.g. randomized clinical trials).
- It consists of a list of research questions and corresponding analyses, ideally with a few comments to explain their rationale.

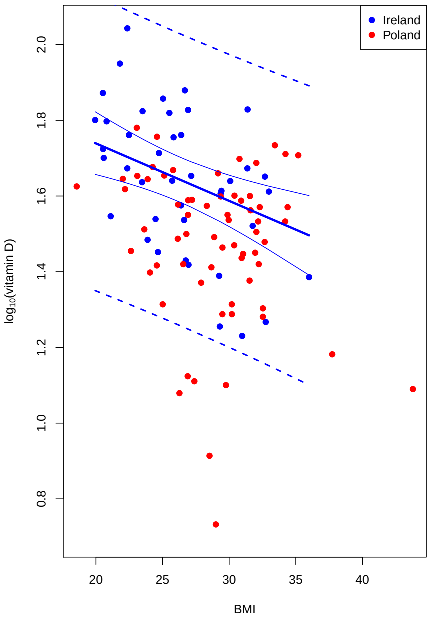
It helps to:

- better discuss with your collaborators and supervisors.
- anticipate challenges (and prevent many stressful situations...)
- rigorously prespecify your analyses and therefore increase the trust that you and your readers can have in your results.
- *“In fact, a lot of questionable research practices can be avoided with a study protocol and statistical analysis plan.”*[12]

It is completely fine to make revisions to the statistical plan and perform post hoc analyses as long as conclusions based on these additional analyses are suitably calibrated.

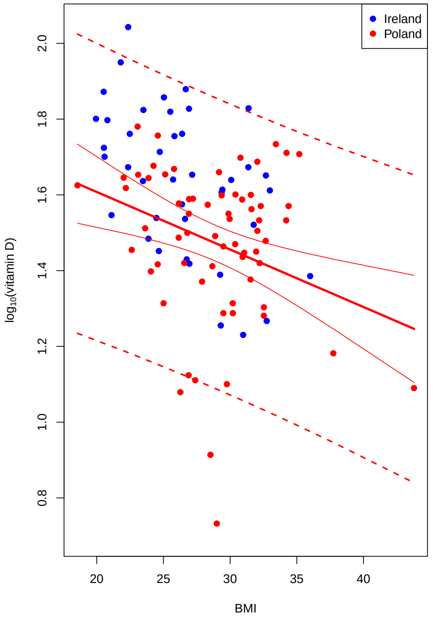
[12] Schwab et al. “Ten simple rules for good research practice.”PLoS Computational Biology" 18.6 (2022): e1010139.

Prediction interval vs confidence intervals



- **Confidence interval** (of the estimated mean value): it quantifies the uncertainty in the estimation of the population mean. It tells us where we are “confident” that the population mean is (plain lines).
- **Prediction interval**: it tells us the range of values that include most (95%) of the observations in the entire population (dashed lines). Its width essentially depends on the estimated standard error of the “error term” σ_ε. It relies strongly on the normal distribution assumption of the “error term”.

Prediction interval vs confidence intervals



- **Confidence interval** (of the estimated mean value): it quantifies the uncertainty in the estimation of the population mean. It tells us where we are “confident” that the population mean is (plain lines).
- **Prediction interval**: it tells us the range of values that include most (95%) of the observations in the entire population (dashed lines). Its width essentially depends on the estimated standard error of the “error term” σ_ε. It relies strongly on the normal distribution assumption of the “error term”.

Outline

The multiple linear model

ILO: to outline what the multiple linear model is about
ILO: to list important methods that are special cases of the model
ILO: to describe the connection with the t-test

Why multiple regression?

ILO: to exemplify when a multiple regression can be better than a univariate analysis
ILO: to describe the connection with ANOVA

ANCOVA and model checking

ILO: to use the ANCOVA and interpret the results
ILO: to evaluate some modeling assumption

Digression: Table-I and the statistical analysis plan (SAP)

ILO: to repeat widely recommended practices in statistics

Interaction and subgroup analysis

ILO: to interpret models with interaction and exemplify their usefulness
ILO: to contrast the use of these models and subgroup analyses

Case: a (first) model with an interaction

- Research questions:
  - Is BMI associated with log-vitamin D in both Irish and Polish elderly women?
  - Is there a different association between log-vitamin D and BMI in Irish and Polish elderly women?
- Predictor variable(s):
  - BMI as a quantitative (continuous) variable
  - Country Ireland / Poland
- Data example: Irish and Poland women, $n = 106\ (41 + 65)$.
- Linear model: $Y_i = \alpha + \beta_1 x_i + \beta_2 z_i + \beta_3 x_i \cdot z_i + \varepsilon_i$

$$x_i = \begin{cases} 1 & \text{if } i \text{ is Polish} \\ 0 & \text{if } i \text{ is Irish} \end{cases} \qquad z_i = \text{BMI of subject } i.$$

The term $\beta_3 x_i \cdot z_i$ models an interaction between x and z.

R code & default output

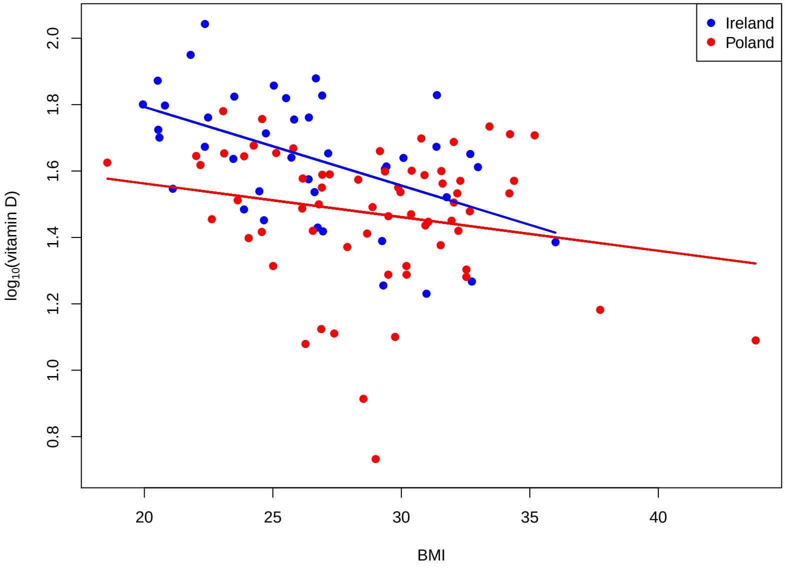
R code:

```
lm5 <- lm(log10(vitd) ~  Country * bmi5, data = irlpolwomen)
summary(irlpolwomen)
```

Output:

```
Coefficients:
                    Estimate Std. Error t value Pr(>|t|)
(Intercept)          2.26626    0.19630  11.545  < 2e-16 ***
CountryPoland       -0.50113    0.25719  -1.948  0.05410 .
bmi5                -0.11834    0.03681  -3.215  0.00175 **
CountryPoland:bmi5   0.06768    0.04650   1.455  0.14865
```

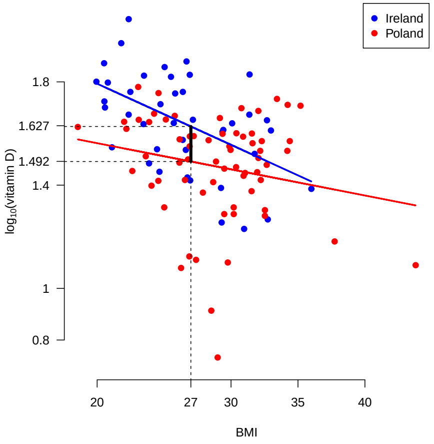
Conclusions?



- Blue slope (Ireland) $\hat{\beta}_2$= -0.11834/5 ≈ -0.12/5
- Red slope (Poland) $\hat{\beta}_2 + \hat{\beta}_3$= (-0.11834 + 0.06768)/5 ≈ -0.05/5
- Difference in slope (Poland - Ireland) $\hat{\beta}_3$= 0.06768/5 ≈ 0.07/5

Trick: re-parametrization for a nicer interpretation of all estimates

We refit the same model after substracting 27 to the BMI variable.



R code:

```
irlpolwomen$bmi5b <- (irlpolwomen$bmi-27)/5
lm5b <- lm(log10(vitd) ~  Country * bmi5b,
           data = irlpolwomen)
summary(lm5b)
```

Output:

```
Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)           1.62722    0.03021  53.862  < 2e-16 ***
CountryPoland        -0.13567    0.03995  -3.396 0.000975 ***
bmi5b                -0.11834    0.03681  -3.215 0.001749 **
CountryPoland:bmi5b   0.06768    0.04650   1.455 0.148649
```

- (Intercept) 1.62722: estimated mean log vitamin D for an elderly Irish woman having a BMI of 27 (i.e. the "reference" woman here). This is an estimated mean.
- CountryPoland -0.13567: we estimate that, in average, the log vitamin D of a Polish elderly woman having a BMI of 27 is 0.13567 lower than that of an Irish elderly woman also having a BMI of 27. This is an estimated difference in means.
- bmi5b -0.11834: we estimate that, in average, the log vitamin D of any elderly Irish woman is 0.11834 lower than that of another elderly Irish woman having a 5-unit lower BMI. This is an estimated difference in means.
- CountryPoland:bmi5b 0.06768: we estimate that, in average, the difference in log vitamin D between two elderly Irish women, one having a 5-unit lower BMI than the other, is 0.06768 larger than the same difference among Polish elderly women. This is an estimated difference in differences in means.

  We further estimate that, in average, the log vitamin D of any elderly Polish woman is 0.11834-0.06768≈ 0.05 lower than that of an elderly Polish woman having a 5-unit lower BMI.

Note: be careful when writing conclusion sentences: are you comparing "A to B" or "B to A" in the sentence? Is it the same in the output of the software?

Trick: changing the reference level (for the slope in the other group)

R code:

```
irlpolwomen$Country2 <- relevel(irlpolwomen$Country,ref="Poland")
lm5c <- lm(log10(vitd) ~  Country2 * bmi5b, data = irlpolwomen)
FormatResLm(lm5c)
```

Output:

```
                      Est 2.5 % 97.5 %  p-value
(Intercept)          1.49  1.44   1.54  < 2e-16
Country2Ireland      0.14  0.06   0.21 0.000975
bmi5b               -0.05 -0.11   0.01 0.077570
Country2Ireland:bmi5b -0.07 -0.16   0.02 0.148649
```

Note: the effect of BMI on log-vitamin D is not significant among Polish elderly women (p-value=0.078). This could not be read from previous R output, although this is interesting for our research question!

Two-way ANOVA with interaction

- Research question: is there a difference in average log-vitamin D between Irish and Polish elderly women having the same BMI group?
- Predictor variable(s):
  - BMI "normal" (18.5-25) / "overweight" (>25).
  - Country Ireland / Poland
- Data example: Irish and Poland women, $n = 106\ (41 + 65)$.
- Linear model: $Y_i = \alpha + \beta_1 x_i + \beta_2 z_i + \beta_3 x_i \cdot z_i + \varepsilon_i$

$$x_i = \begin{cases} 1 & \text{if } i \text{ is Polish} \\ 0 & \text{if } i \text{ is Irish} \end{cases} \qquad z_i = \begin{cases} 1 & \text{if } i \text{ is "overweight"} \\ 0 & \text{if } i \text{ has a "normal" weight} \end{cases}$$

This is a two-way ANOVA model with interaction.

Parameters interpretation

According to the model, the means of log-vitamin D are:

Country BMI	Ireland	Poland
"Normal"	α	$\alpha + \beta_1$
"Overweight"	$\alpha + \beta_2$	$\alpha + \beta_1 + \beta_2 + \beta_3$

- α: mean outcome for Irish with "normal" BMI (**reference group**).
- β_1: difference in mean outcome between Irish and Polish among women with "normal" BMI.
- β_2: difference in mean outcome between women with "overweight" and those having a "normal" BMI, among Irish women.
- β_1 $+\beta_3$: difference in mean outcome between Irish and Polish among women with "overweight".
- β_2 $+\beta_3$: difference in mean outcome between women with "overweight" and those having a "normal" BMI, among Polish women.
- β_3: difference in differences in means....

R code:

```
lm6 <- lm(log10(vitd) ~  Country * bmigroup, data = irlpolwomen)
summary(lm6)
```

Output:

```
Coefficients:
                         Estimate Std. Error t value Pr(>|t|)
(Intercept)               1.71987    0.04840  35.538   <2e-16 ***
CountryPoland            -0.12142    0.07393  -1.643   0.1036
bmigroup1                -0.12682    0.06198  -2.046   0.0433 *
CountryPoland:bmigroup1 -0.02838    0.08758  -0.324   0.7466
```

Conclusions? Does this output answer our research question?

[13] Of course, we would like to also see the 95%-CIs, for complete reporting of the results. This will able us to distinguish between the "statistical" and "clinical" importance of the difference we observe.

R code:

```
lm6 <- lm(log10(vitd) ~  Country * bmigroup, data = irlpolwomen)
summary(lm6)
```

Output:

```
Coefficients:
                         Estimate Std. Error t value Pr(>|t|)
(Intercept)               1.71987    0.04840  35.538   <2e-16 ***
CountryPoland            -0.12142    0.07393  -1.643   0.1036
bmigroup1                -0.12682    0.06198  -2.046   0.0433 *
CountryPoland:bmigroup1 -0.02838    0.08758  -0.324   0.7466
```

Conclusions? Does this output answer our research question?

In part yes, but not fully. We see both the estimated difference and the p-value for the difference between the countries, for women with "normal" BMI only. We do not see these results (no p-value) for those with "high" BMI. [13]

[13] Of course, we would like to also see the 95%-CIs, for complete reporting of the results. This will able us to distinguish between the "statistical" and "clinical" importance of the difference we observe.

We re-fit the model after changing the reference group for the BMI group variable.

R code:

```
irlpolwomen$bmigroup2 <- relevel(irlpolwomen$bmigroup,ref="1")
lm6b <- lm(log10(vitd) ~  Country * bmigroup2, data = irlpolwomen)
summary(lm6b)
```

Output:

```
Coefficients:
                          Estimate Std. Error t value Pr(>|t|)
(Intercept)                1.59305    0.03872  41.147  < 2e-16 ***
CountryPoland             -0.14981    0.04697  -3.190  0.00189 **
bmigroup20                 0.12682    0.06198   2.046  0.04330 *
CountryPoland:bmigroup20  0.02838    0.08758   0.324  0.74656
```

Conclusions?

95%-CI and conclusion sentences

R code:

```
round(confint(lm6b),2)
```

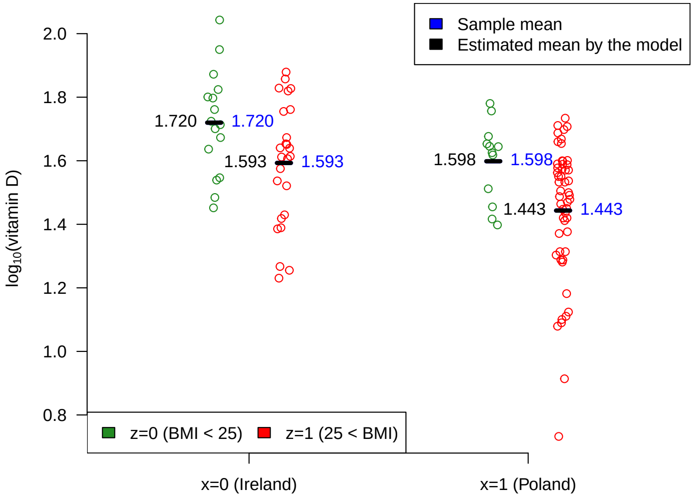
Output:

```
                         2.5 % 97.5 %
(Intercept)               1.52   1.67
CountryPoland            -0.24  -0.06
bmigroup20                0.00   0.25
CountryPoland:bmigroup20 -0.15   0.20
```

- We estimate that, on average, Polish "overweight" women have a value of $\log_{10}$ vitamin D concentrations 0.15 lower than Irish "overweight" women (95%-CI=[0.06,0.24], p=0.002).
- We did not find evidence that that, on average, Polish "normal weight" women have a value of $\log_{10}$ vitamin D concentrations different to that of Irish "normal weight" women (Mean Difference= -0.12, 95%-CI=[-0.27,0.03], p=0.104).[14]
- Note: we computed two p-values, thus adjusting for multiple testing might be needed.[15]

[14] One needs to run `round(confint(lm6),2)` to read the confidence interval for this "normal weight" BMI group.

[15] e.g. typically needed in the context of confirmatory research, if this is the main analysis. Typically not needed when this is a posthoc / supplementary/ exploratory analysis.



- Here the estimated means are equal to the sample means. We say that the model for the mean is saturated (because we have 4 parameters to estimate 4 means).
- We note the smaller sample size for "normal weight" women. We can hypothesize that the non-significant result in that group is due to lack of power.

Interaction versus subgroup analysis

- In the two previous examples, the only difference in the model assumptions between using a model with an interaction and performing a subgroup analysis (one per country) is the way we model the standard deviation of the error term σ_ε: we would model two different values with the subgroup analysis, whereas only one with the interaction model.
- If we had adjusted on more variables, then the difference would be more important, because the subgroup analysis would implicitly also model interactions with all these other variables.

Case: stratifying vs adjusting with interaction

Comparing estimated parameters:

	Statistical analysis choice			
	Adjust + inter		Subgroup	
	Poland	Ireland	Poland	Ireland
BMI (by 5)	-0.126	-0.050	-0.103	-0.047
Sun: sometimes vs avoid	0.020	0.020	-0.068	0.073
Sun: prefer vs avoid	0.054	0.054	-0.117	0.159

From the three models:

1. `lm(log10(vitd) ~ Country * bmi5b + sunexp, data = irlpolwomen)`
2. `lm(log10(vitd) ~ bmi5b + sunexp, data = poland)`
3. `lm(log10(vitd) ~ bmi5b + sunexp, data = ireland)`

Note: in model 1 (adjust + interaction), we assume that the "effect" of sun exposure is similar in Poland and Ireland, which is not the case with the subgroup analysis.

Final words on modeling

Many topics discussed today and on day 6 are important beyond the linear and logistic model.

Most of the reasoning about modeling choices, including:

- which variables to include?
- how? (with or without interaction, categorized version or not...)
- why does it matter?

This applies for more complicated model that you may encounter/need during your research career.

When, why, where to seek statistical help?

When? if you are not sure about how to...

- plan your experiment or clinical trial
- analyze your data
- answer reviewers or collaborators concerns

(this might be more complicated than it appears)

Why?

- you might get quick help and simple advice that make a big difference.
- to minimize the risk of "wasting" your precious research time and work by inappropriately analysis of your data.
- why not? it can sometimes be free of charge :-)

Where?

- Section of Biostatistics (https://publichealth.ku.dk/about-the-department/biostat/)
  - by phone (quick & simple questions): free of charge
  - short meeting (20 mins): free of charge
  - new collaborations: sometimes free, but usually not.
- private companies also exist.