

# BIOSTATISTICS WITH

SUMMER WORKSHOP  
(MITGEST network)

24-27 JULY 2024

*Maria Chiara Mimmi, PhD*

# WORKSHOP SCHEDULE

- 4 days
  - 1. Intro to R and data analysis
  - 2. Statistical inference & hypothesis testing
  - 3. Modeling correlation and regression
  - 4. Machine Learning; MetaboAnalyst; Power Analysis
- Each day will include:
  - Frontal class (MORNING)
  - Practical training with R about the topics discussed in the morning. (AFTERNOON)

# DAY 3 – LECTURE OUTLINE

- Testing and summarizing relationship between 2 variables (**correlation**)
  - Pearson's analysis (param)
    - 2 numerical variables
  - Spearman test (no param)
    - 2 numerical variables (non linear relationships)
- Measures of **association**
  - Chi-Square test of independence
    - 2 categorical variables
  - Fisher's Exact Test
    - alternative to the Chi-Square Test of Independence
- From correlation/association to **prediction/causation**
  - The purpose of observational and experimental studies
- Widely used analytical tools
  - Simple linear regression models
  - Multiple Linear Regression models
- Shifting the emphasis on **empirical prediction**
  - Introduction to Machine Learning (ML)
  - Distinction between Supervised & Unsupervised algorithms

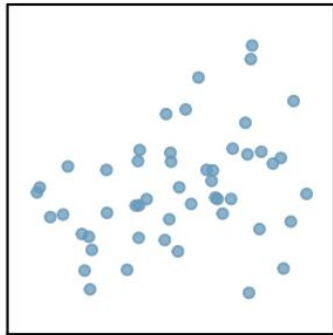
# Summarizing relationships between two variables

Correlation

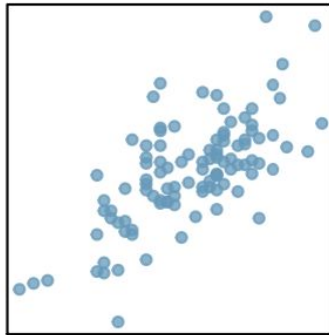
# Defining correlation

- **Correlation** is a numerical summary statistic that measures the *strength of a linear relationship* between two variables
  - denoted by **r** (correlation coefficient) which takes values **between -1 and 1**

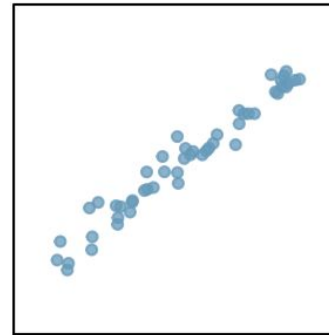
positive  
correlation



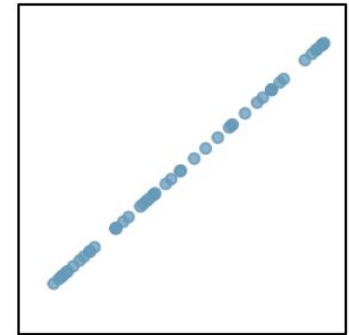
$R = 0.33$



$R = 0.69$

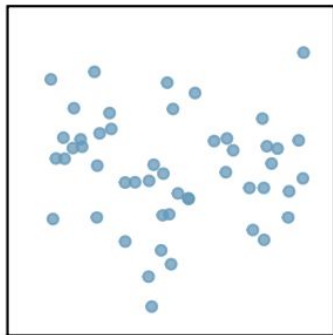


$R = 0.98$

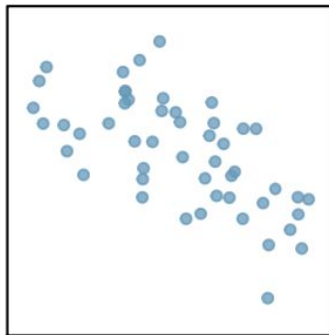


$R = 1.00$

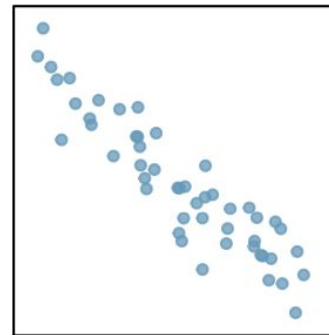
negative  
correlation



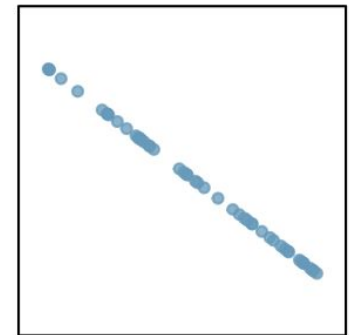
$R = -0.08$



$R = -0.64$



$R = -0.92$



$R = -1.00$

Source: Vu, J., & Harrington, D. (2021). *Introductory Statistics for the Life and Biomedical Sciences*. Retrieved from <https://www.openintro.org/book/biostat/>

# Most used measures of correlation

Correlation coefficient	Type of relationship	Levels of measurement	Data distribution
<b>Pearson's <math>r</math></b> ( for population)	Linear	Two <b>quantitative</b> (interval or <b>ratio</b> ) variables	Normal distribution
<b>Spearman's</b> ( for population)	Non-linear	Two <b>ordinal, interval or ratio</b> variables	Any distribution
<b>Cramér's <math>V</math></b> (Cramér's $\phi$ )	Non-linear	Two <b>nominal</b> variables	Any distribution
<b>Kendall's (<math>\tau</math>)</b>	Non-linear	Two <b>ordinal, interval or ratio</b> variables	Any distribution

# What is the link between correlation and covariance?

- **Covariance** is another helpful statistic that **tells whether both variables vary in the same direction** (positive covariance) **or in the opposite direction** (negative covariance)
  - Unlike in correlation, **there is no meaning of covariance numerical value only sign is useful**
  - $is > 0$  --> vary in the same direction
  - $is < 0$  --> vary in the opposite direction
  - $is \sim 0$  --> vary independently from each other
- The general formula for **Covariance** is:
  - where  $s_x$  is the standard deviation of x and  $s_y$  is the standard deviation of y
  - dividing Covariance by  $s_x s_y$ , we obtain Correlation **r** with range [-1, +1]
- Interesting to note that:

# Correlation between 2 numerical variables

Pearson's correlation (parametric test)



# Pearson's correlation

**Pearson correlation** ( $r$ ) measures a linear association between 2 CONTINUOUS variables ( $x$  and  $y$ ) or 2 dichotomous variables

- It's also known as a parametric correlation test because it depends to the distribution of the data.
- The Pearson correlation evaluates the linear relationship between two continuous variables.

## FORMULA

WHERE:

$x$  and  $y$  are two vectors of length  $n$

$\bar{x}$  and  $\bar{y}$  correspond to the means of  $x$  and  $y$ , respectively.

We can test the statistical significance of the correlation statistic as well.

The p-value (significance level) of the correlation can be determined by calculating

with

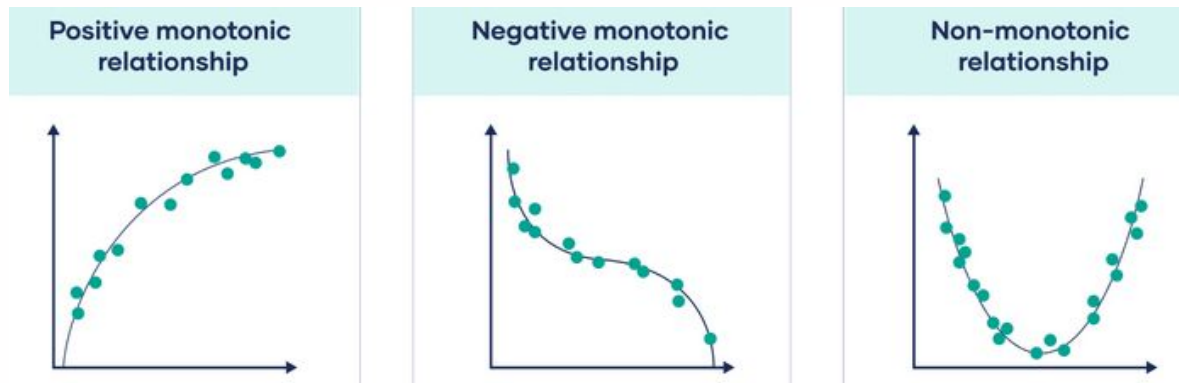
# Correlation between 2 numerical variables

Spearman's correlation (non parametric test)

# Spearman's rank order correlation coefficient

Spearman's correlation (  $r_s$  ) is a **nonparametric alternative to Pearson's correlation**, used for

- continuous data with a **non linear, monotonic** relationships, or
- **ordinal** data (e.g. Likert scale survey questions: *strongly agree, agree, etc.*)



## FORMULA

- where:
  - $r_s$  is Spearman's coefficient of rank correlation.
  - $d_i$  is the difference between the ranks for each pair.
  - $n$  is the number of paired observations.

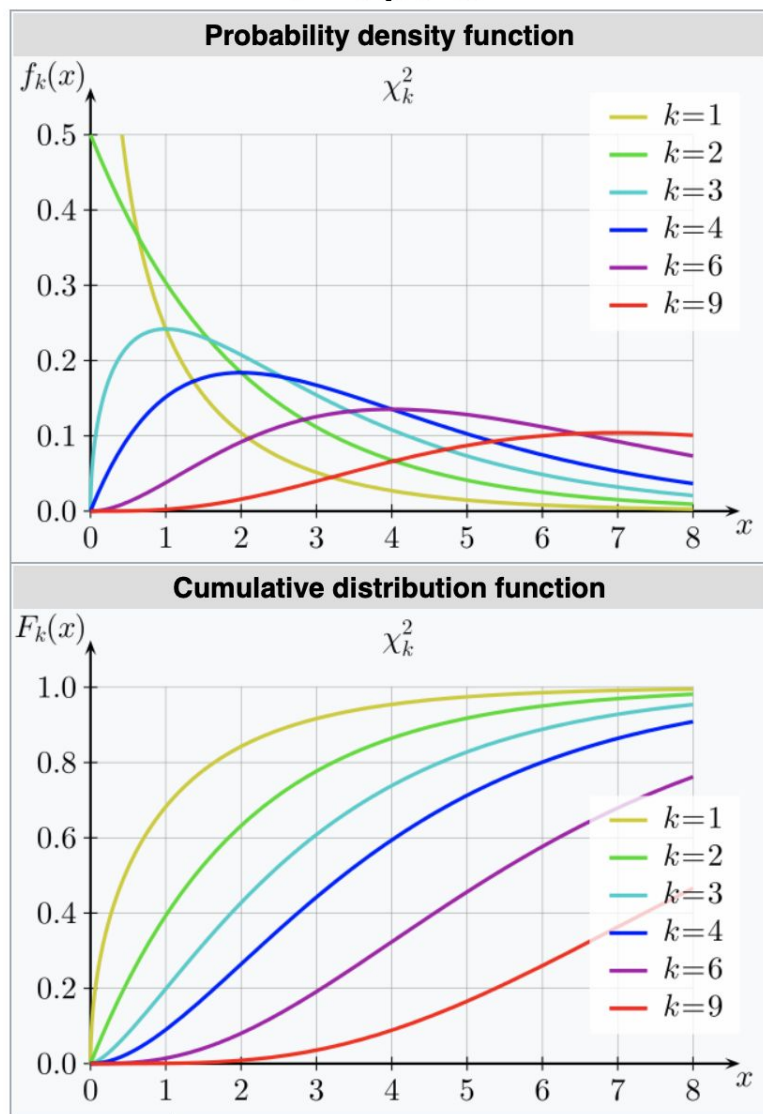
Hypothesis Test: Rank Correlation

# Chi Squared Distributions

A widely used analytical tool

# The chi-squared distribution

## chi-squared



- The **chi-squared distribution** () is a family of continuous probability distributions
- It results from the sum of squares of  $k$  normally distributed random variables, where  $k$  is the number of degrees of freedom ( $df$ )
- The **mean** is equal to the  $df$  and the **variance** is equal to  $2 \times df$

<b>Notation</b>	$\chi^2(k)$ or $\chi_k^2$
<b>Parameters</b>	$k \in \mathbb{N}^*$ (known as "degrees of freedom")
<b>Support</b>	$x \in (0, +\infty)$ if $k = 1$ , otherwise $x \in [0, +\infty)$
<b>PDF</b>	$\frac{1}{2^{k/2} \Gamma(k/2)} x^{k/2-1} e^{-x/2}$
<b>CDF</b>	$\frac{1}{\Gamma(k/2)} \gamma\left(\frac{k}{2}, \frac{x}{2}\right)$
<b>Mean</b>	$k$
<b>Median</b>	$\approx k \left(1 - \frac{2}{9k}\right)^3$
<b>Mode</b>	$\max(k - 2, 0)$
<b>Variance</b>	$2k$

# Applications of the Chi-Square Test

- Unlike the **Normal distribution**, very few real-world observations follow a **chi-square distribution**, but it is used extensively in hypothesis testing (also due to its close relationship with the normal).
  - As **k** increases, the distribution looks more and more similar to a normal distribution
- The **Chi-square test** helps to answer the following questions:
  - 1. Independence test**
    - Are two categorical variables independent of each other?
      - for example, does gender have an impact on whether a person has a Netflix subscription or not?
  - 2. Distribution (or Goodness of fit) test**
    - Are the observed values of two categorical variables equal to the expected values?
      - One question could be, is one of the three video streaming services Netflix, Amazon, and Disney subscribed to above average?
  - 3. Homogeneity test**
    - Are two or more samples from the same population?
      - One question could be whether the subscription frequencies of the three video streaming services Netflix, Amazon and Disney differ in different age groups.

# Correlation between 2 categorical variables

Chi Squared test of independence

# A useful tool for categorical variables: contingency tables

- A **contingency table** summarizes data for 2 categorical variables (each value in the table representing the times a particular combination of outcomes occurs)
- Below we see 2 categorical variables “**gender**” (male, female) and “**has Netflix subscription**” (yes, no)

Frequency			SUM
	Male	Female	
Netflix yes	10	13	<b>23</b>
Netflix no	15	14	<b>29</b>
<b>SUM</b>	<b>25</b>	<b>27</b>	

- The **row totals** (counts across each row) and the **column totals** (counts across each column) are the **marginal totals**
- Frequencies can also be shown as proportions



# Computing the Chi-Square Test of Independence

- E.g. suppose we are testing the independence of the two categorical variables “**gender**” (male, female) and “**has Netflix subscription**” (yes, no)
- The test performs a **comparison** of these two contingency tables:

<u>Observed</u> Frequency		
	Male	Female
Netflix yes	10	13
Netflix no	15	14

<u>Expected</u> Frequency		
	Male	Female
Netflix yes	$(23 \times 25) / 52 = 11.06$	$(23 \times 27) / 52 = 11.94$
Netflix no	$(29 \times 25) / 52 = 13.94$	$(29 \times 27) / 52 = 15.06$

## IMPORTANT ASSUMPTIONS TO NOTICE:

- The assumption for the **chi-squared ()** test statistic is that the expected frequencies per cell are  $> 5$
- The **chi-squared ()** test uses only the categories but NOT rankings

# Computing the Chi-Square Test of Independence (computation)

- Let's compute the example for the two variables “gender” and “has Netflix subscription”

Observed Frequency		
	Male	Female
Netflix yes	10	13
Netflix no	15	14

Expected Frequency		
	Male	Female
Netflix yes	$(23 \times 25) / 52 = 11.06$	$(23 \times 27) / 52 = 11.94$
Netflix no	$(29 \times 25) / 52 = 13.94$	$(29 \times 27) / 52 = 15.06$

- The **chi-squared ()** test statistic is calculated via:
- where:
  - observed frequency and
  - Expected frequency**
    - calculated for each cell in the contingency table
- The test assumptions are:
  - $H_0$ : (null hypothesis) The two variables are independent.
  - $H_1$ : (alternative hypothesis) The two variables are not independent. (i.e. they are associated)
  -

# Interpreting the Chi-Square Test of Independence

- The **chi-squared ()** test statistic calculated value:
- BY THE CRITICAL REGION: Looking at the distribution, for a significance level of 5% and a *df* of 1, the **critical chi-squared value = 3.841**
  - → Since the **calculated chi-squared value=0.35** is smaller, we **FAIL TO REJECT the null** ( $H_0$ : *The two categorical variables are independent*)
- BY THE p VALUE: Also, the **p-value** associated to the and is **0.5541**.
  - → Since this p-value is not less than 0.05, we fail to reject the null hypothesis.
- This means we do not have sufficient evidence to say that there is an association between gender and political having a Netflix account!

# Chi Squared test (another application)

Goodness of Fit Test for one categorical variable

# Chi-Square Goodness of Fit Test

- GOAL: a **Chi-Square goodness of fit test** is used to **determine whether or not a categorical variable follows a hypothesized distribution**.
  - With **high** goodness of fit, the values expected based on the model are **close to** the observed values
  - With **low** goodness of fit, the values expected based on the model are **far from** the observed values
- EXAMPLES OF APPLICATION:
  - *Is this sample drawn from a population with 90% right-handed and 10% left-handed people?*
  - *Do offspring have with an equal probability of inheriting all possible genotypic combinations (i.e., unlinked genes)?*
- HYPOTHESIS FORMULATION
  - Null Hypothesis ( $H_0$ ): The population follows the specified distribution.
  - Alternative Hypothesis ( $H_a$ ): The population does not follow the specified distribution.

# Chi-Square Goodness of Fit Test (computation)

- FORMULA: The formula is essentially the same as in the independence test
- where  $O_i$  = Observed Frequencies and  $E_i$  = Expected Frequencies
- ... with  $k$  (number of groups minus 1)
- WHEN SHOULD WE USE IT? (assumptions)
  1. We are testing the distribution of **one categorical variable**
    - if you have a continuous variable, it should be converted to categorical (this is called *data binning*) or a different test can be used (like the Kolmogorov–Smirnov goodness of fit test for continuous variables)
  2. The sample was randomly selected from the population.
  3. There are a minimum of 5 observations expected in each group.

# Chi-Square Goodness of Fit Test (example)

- GOAL: examine the appropriateness of hypothesized distribution for a dataset
- CASE: In the FAMuSS study (we'll see later in the lab) volunteers were observed at a university, so we test if their distribution by categorical variable **race** is the same as (i.e. *representative of*) the general US population?

Race	African.American	Asian	Caucasian	Other	Total
FAMuSS (Observed)	27	55	467	46	595
US Census (Expected)	76.16	5.95	478.38	34.51	595

- where  $O_i$  = Observed Frequencies and  $E_i$  = Expected Frequencies
- ... with  $k$  (number of groups minus 1)
- The  $\chi^2$  statistic is extremely large, and the associated p-value  $< 0.001 \rightarrow$
- We **reject the null hypothesis** (= the sample proportions should equal the population proportions)... in fact, we can see for example the higher Asian representation in sample

# Correlation between 2 categorical variables - Fisher's Exact Test

(alternative to the Chi-Square Test of  
Independence)



# Fisher's Exact Test

- Fisher's Exact Test is used to determine whether or not there is a significant association between two categorical variables.
- It is typically used as an alternative to the Chi-Square Test of Independence when one or more of the cell counts in a 2×2 table is less than 5.
- Fisher's Exact Test uses the following null and alternative hypotheses:
  - $H_0$ : (null hypothesis) The two variables are independent.
  - $H_1$ : (alternative hypothesis) The two variables are not independent.

# Calculate **effect size** after a Chi-Square Test

3 alternatives to assess “strength” of the association (if any)

# Three Ways to Calculate Effect Size for a Chi-Square Test

- So, we have seen 2 commonly used **Chi-Square tests**:
  - **Chi-Square Test for Independence**: Used to determine whether or not there is a significant association between two categorical variables from a single population.
  - **Chi-Square Test for Goodness of Fit**: Used to determine whether or not a categorical variable follows a hypothesized distribution
- For both of these tests, we obtain a **p-value** that tells us “if” an association is found (i.e. we should reject the null hypothesis of the test or not).
- Then, we may wonder about the **effect size** of the test (i.e. “how strong” an association is)
- There are 3 ways to measure **effect size**:
  1. **Phi** ( $\phi$ )
    - for 2 x 2 contingency table
  2. **odds ratio** (OR)
    - for 2 x 2 contingency table
  3. **Cramer's V** (V)
    - for larger tables
      - example in lab

# Correlation between... 1 numerical variable and 1 categorical variables

... we have actually met before 🤔

# Correlation between 1 numerical variable and 1 categorical variables

- Recall that we have already encountered methods for for comparing **numerical** data across groups in the previous lessons
  1. Using **side-by-side boxplots** for visual comparison of how the distribution of a numerical variable differs by category
  2. Using **One-Way ANOVA** for testing relationships between Numerical and Categorical variables
    - i.e. the extension of the t-test for more than 2 groups

# From correlation/association to prediction/causation

The purpose of observational studies v.  
experimental studies

# From Correlation/Association to Prediction/Causation → experimental studies

- So far, we worked on “**observational studies**” (i.e. data where you have not controlled the *assignment of the treatment*) measuring variables of interest
  - even if we may find CORRELATION OR ASSOCIATION, but it DOES NOT IMPLY CAUSATION!
  - WHY? there can be “**hidden variables**” that affect the relationship between the explanatory variable and the response variable
- “**Experimental studies**” help us studying **causation** in that they are “*designed to provoke a response*”
  - they involve *assigning the treatment* to an *experimental unit* (or subject) and observing its *effect*
  - they follow some design PRINCIPLES that provide robust evidence for causation

# A conceptual framework to understand different types of statistical **modeling** (part 1/2)

## 1. **association/correlation** → observational studies

- aimed at **summarizing or representing the data structure**, without an underlying causal theory
- may help **form hypotheses** for explanatory and predictive modeling

## 2. **causal explanation** → experimental studies

- aimed at **testing “explanatory connection”** between treatment and outcome variables
- prevalent in “**causal theory-heavy**” fields (like: economics, **psychology**, environmental science, etc.)

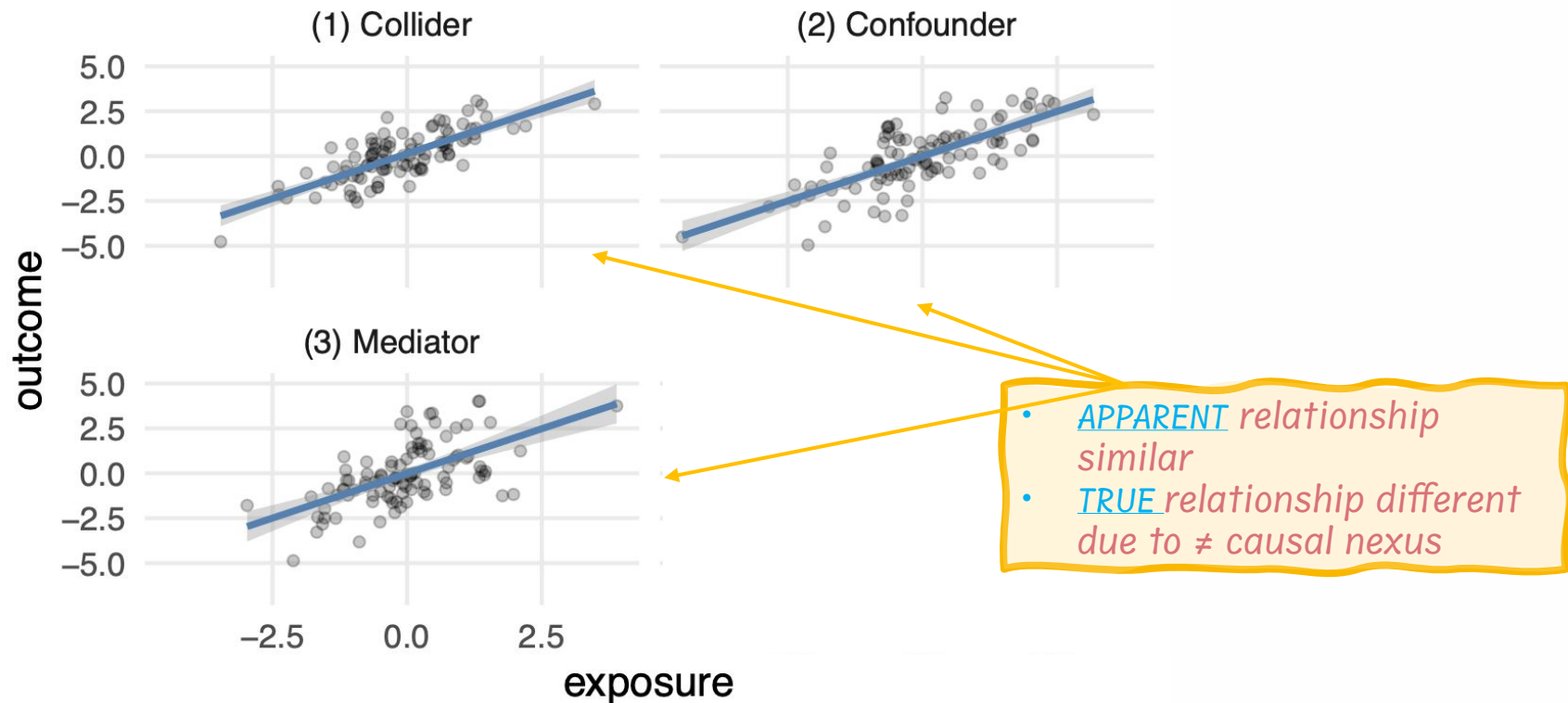
### • **NOTE:**

- ✓ The **same modeling approach** (e.g. fitting a regression model) can be used for **different goals**
- ✓ While they shouldn't be confused, **explanatory power** and **predictive accuracy** are complementary goals: e.g. in bioinformatics (which has little theory and abundance of data), predictive models are pivotal in generating avenues for causal theory.



# Typical challenges in estimating causal effects: visual intuition

- Consider 3 distinct datasets: while their statistical summaries and visualizations are very similar, the **true causal effect differs!**
- Deciding the** correct model requires knowledge of the data-generating mechanism (i.e. the random assignment to exposure/not exposure in experiments)



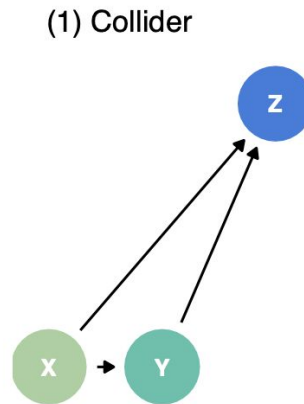
Source: Barrett, M., McGowan, L. D., & Gerke, T. (2024). *Causal Inference in R*. Retrieved from <https://www.r-causal.org/>

# Typical challenges in estimating causal effects: visual intuition

- Directed acyclic graphs (DAGs) can offer visual intuition of the causal nexus at play in the 3 datasets. Failure to adjust models to these situation leads to **BIAS**
  - $X$  is some continuous exposure of interest,  $Y$  a continuous outcome, and  $Z$  a known, measured factor

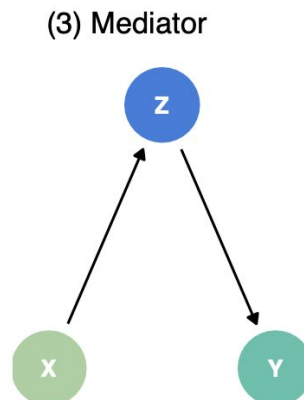
(1) a “**COLLIDER**” is caused by both  $X$  and  $Y$  (it inadvertently connects the 2). E.g.:

- $X$  = sodium intake
- $Y$  = systolic blood pressure
- $Z$  = urinary protein excretion

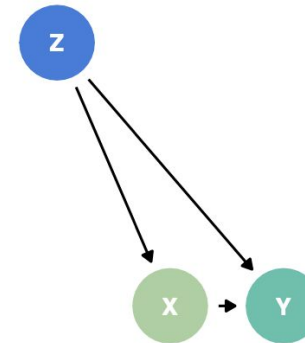


(3) a “**MEDIATOR**” is caused by  $X$  and then it causes  $Y$ . E.g.:

- $X$  = screen time
- $Y$  = obesity
- $Z$  = physical exercise



(2) Confounder



(2) a “**CONFOUNDER**” causes both  $X$  and  $Y$ . E.g.:

- $X$  = smoking
- $Y$  = lung cancer
- $Z$  = alcohol (consumers also tend to be smokers)

we'll revisit this later in multivariate regression...

Source: Barrett, M., McGowan, L. D., & Gerke, T. (2024). *Causal Inference in R*. Retrieved from <https://www.r-causal.org/>

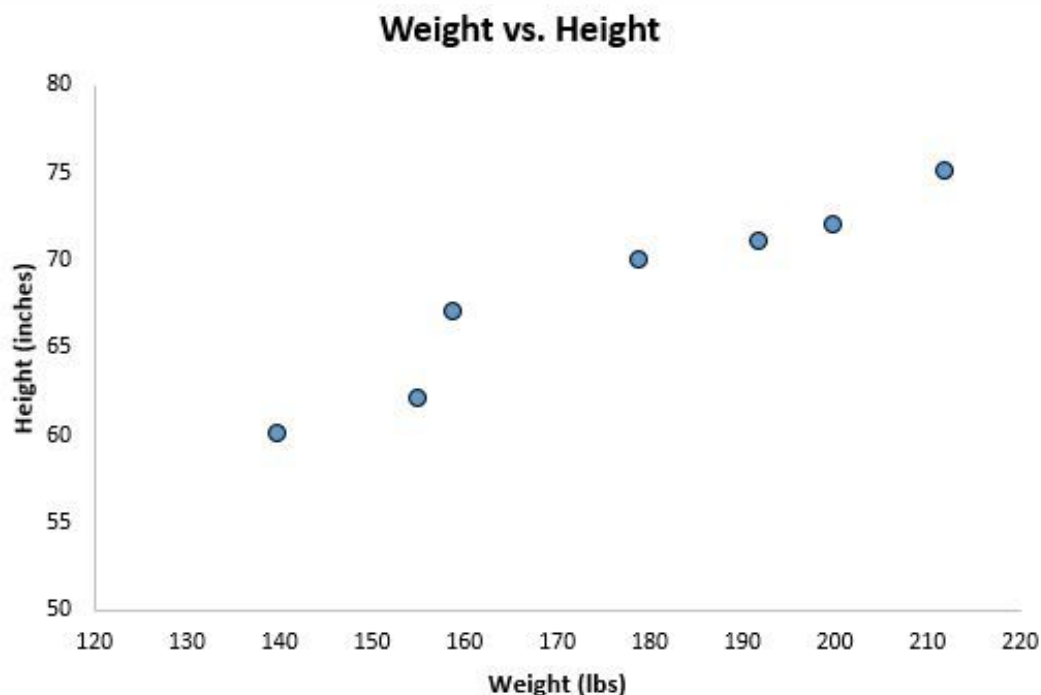
# Simple Linear Regression

Regression analysis is a widely used method  
for **prediction** and – *given the proper  
experimental conditions* – for **causal  
explanation**

# Simple linear regression: example

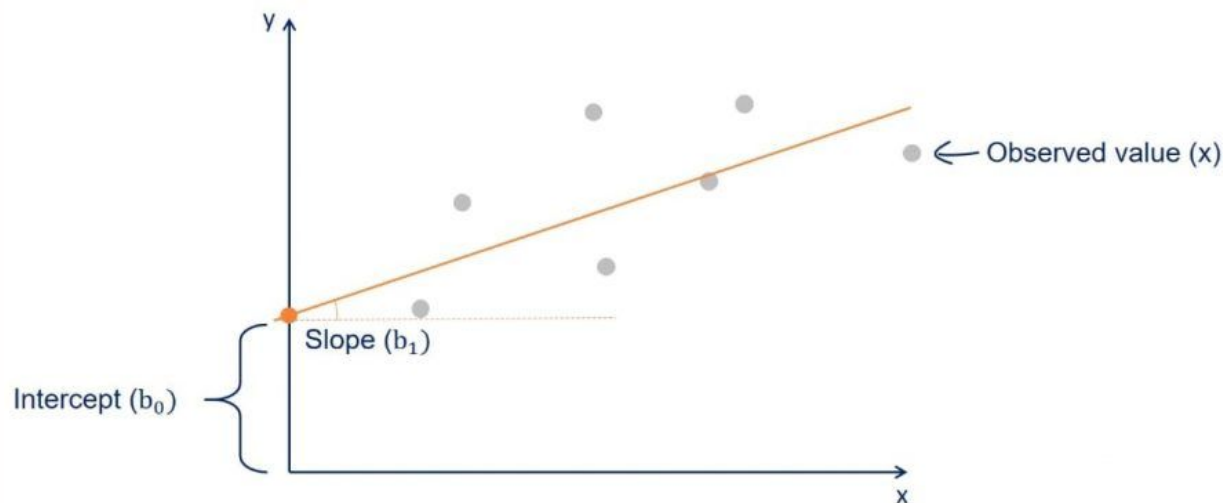
- Regression models are highly valuable, as they are one of the most common ways to make inferences and predictions
- Linear regression is OK with data that exhibit linear or approximately linear relationships
- **Simple linear regression** is a statistical method you can use to understand the relationship between two variables, (the **predictor variable**) and (the **response variable**)

Weight (lbs)	Height (inches)
140	60
155	62
159	67
179	70
192	71
200	72
212	75



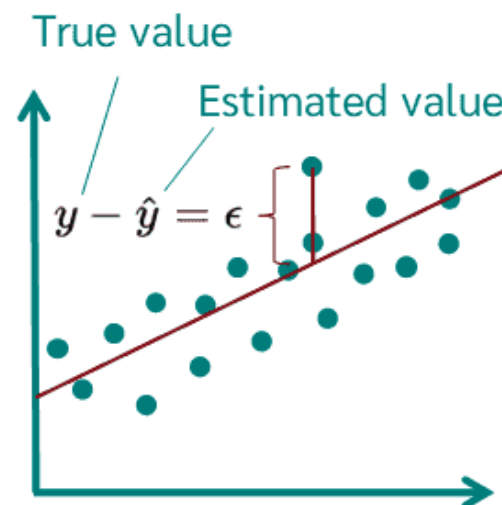
# Functional (linear) relationship and regression

- The **correlation coefficient** gave us information about the degree to which points (corresponding to  $x$  and  $y$  pairs) were clustered around a straight line ... but nothing about the **slope** of that line
- **regression analysis**, instead, provides this kind of information:
  - we want to know exactly how those 2 variables are related
  - (given we hypothesized a linear relationship) the model has a **functional form** that provides an **intercept** and a **slope**:



# Linear regression (Ordinary Least Square)

- The **OLS regression line** is chosen as to minimize the difference between estimated values and actual ones
  - in fact, OLS seeks the minimum sum of squared distances between each point and the regression line
  - it is the “best fitting” line given any data of points
- **NOTE:** like with previous **inferential statistics methods**, we are making statements on the **population** of interest based on some **sample** data available



Population data of interest	<u>Sample data we have</u>
= <b>true</b> Y values (dependent/response variable)	= <b>estimated</b> (or predicted) Y values based on X values
= <b>true</b> X values (independent/explanatory variable)	= <b>sample</b> X values
= <b>true</b> intercept	= <b>estimated</b> intercept
= <b>true</b> slope/coefficient on x	= <b>estimated</b> slope/coefficient on x
= <b>true</b> residual or unobserved part of y	= <b>estimated</b> residual (error), or unobserved part of Y

# OLS Linear regression interpretation

- The formula for the line of best fit is written as:
$$\hat{y} = \beta_0 + \beta_1 x$$
  - where  $\hat{y}$  is the predicted value of the response variable (**height**),  $\beta_0$  is the **y-intercept**,  $\beta_1$  is the **regression coefficient**, and  $x$  is the value of the predictor variable (**weight**).
- For example, in the case of :
$$\hat{y} = 32.7830 + 0.2001x$$
  - $\beta_0 = 32.7830$ . This means **when the predictor variable **weight** is 0 pounds, the predicted **height** is 32.7830 inches.**
    - Sometimes the value for  $\beta_0$  can be useful to know, but not in this specific example
  - $\beta_1 = 0.2001$ . This means that for a **one unit increase in the variable, the variable is predicted to increase(decrease) by 0.2001 units.** Here, a one pound increase in **weight** is associated with a 0.2001 inch increase in (**expected height**), on average.
    - NOTE: just like with previous hypothesis testing on sample means etc., we are testing the coefficients ( $\beta_0$  and  $\beta_1$ ) for statistical significance under  $H_0$ : the coefficient = 0

# Assumptions of linear regression

- For the results of a linear regression model to be valid and reliable, we need to check that the following four assumptions are met:
  1. **Linear relationship:** There exists a linear relationship between the independent variable,  $x$ , and the dependent variable,  $y$
  2. **Normality:** The residuals of the model are normally distributed.
    - Check normality (OF RESIDUALS) with the known methods (QQplot, Shapiro-Wilk, Kolmogorov Smirnov)
  3. **Homoscedasticity:** The residuals have constant variance at every level of  $x$ .
  4. **Independence:** The residuals are independent. In particular, there is no correlation between consecutive residuals in time series data.
    - This is mostly relevant when working with time series data. Ideally, we don't want there to be a pattern among consecutive residuals.



# Diagnostic plotting: residuals

A **residual** is the vertical distance between a data point and the regression line.  $y_i - \hat{y}_i$

- $y_i$ : The **actual response** value for the  $i$ th observation
- $\hat{y}_i$ : The **predicted response** value based on the multiple linear regression model

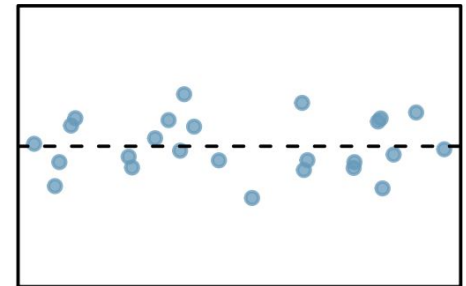
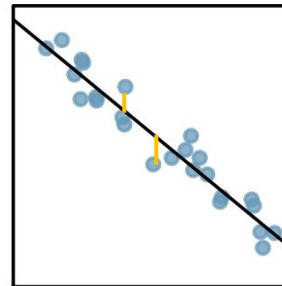
*We want to see a residual plot where data shows random scatter above and below the horizontal line*

In the example on the right:

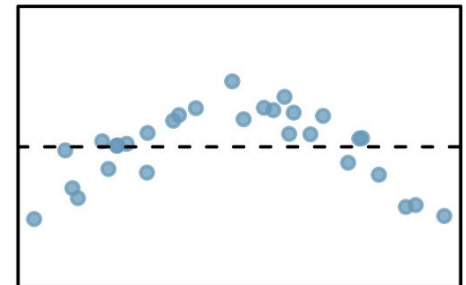
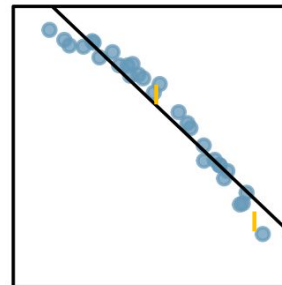
- **Case 1)** linear model is a **particularly good fit!**
- **Case 2)** the original data cycles below and above the regression line
- **Case 3)** the variability of the residuals is not constant; the residuals are slightly more variable for larger predicted values.

Best fitting  
line

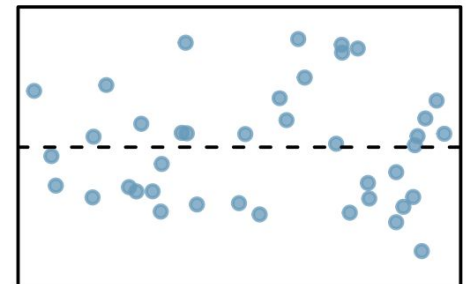
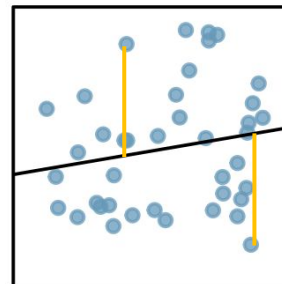
(corresponding)  
Residual plots



1



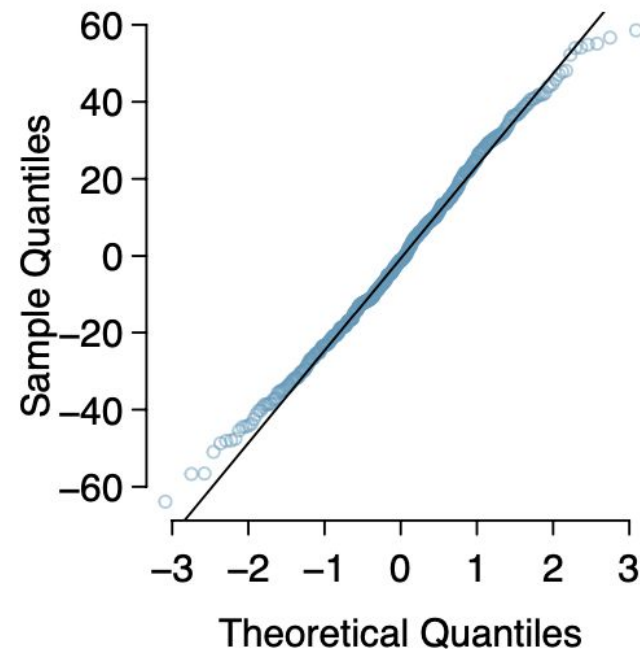
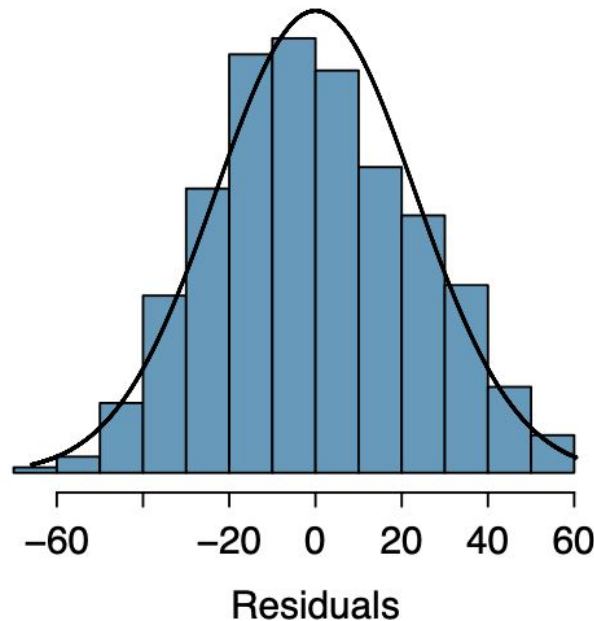
2



3

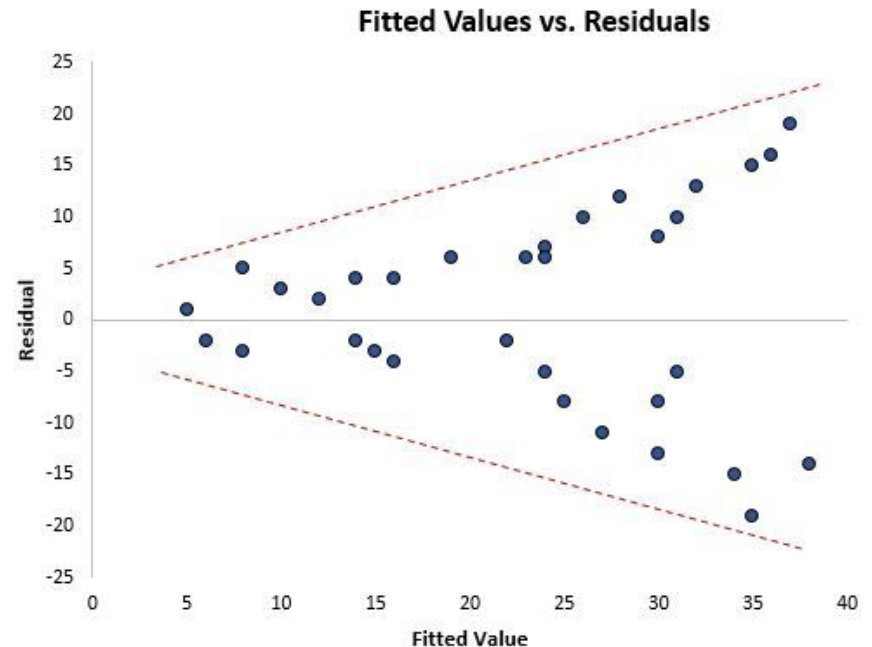
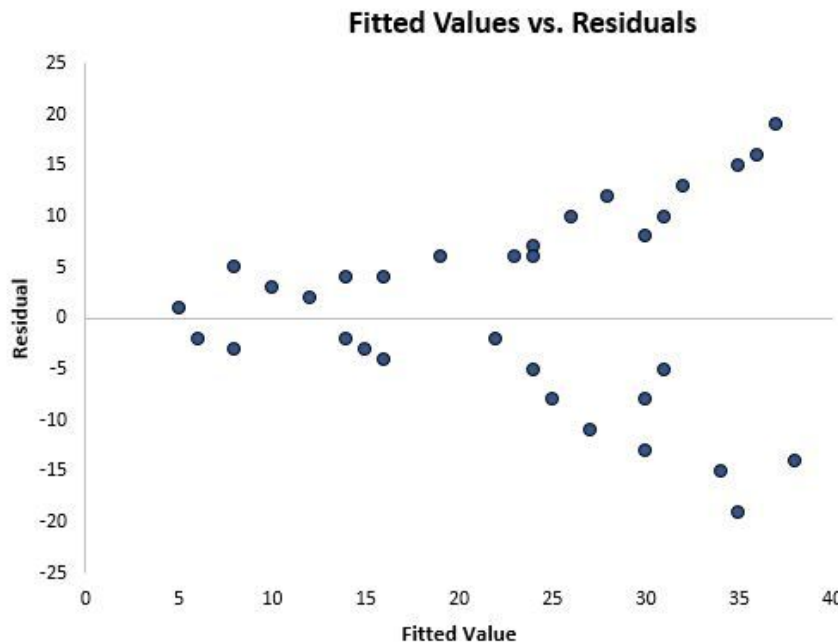
# Diagnostic plotting: normality of residuals

- The residuals of the model are normally distributed.
- Check normality (OF RESIDUALS) with the known methods (QQplot, Shapiro-Wilk, Kolmogorov Smirnov)



# Diagnostic plotting: Homoscedasticity

- ASSUMPTION: The residuals (i.e. the error term) have constant variance at every level of  $x$  (“homoscedasticity”)
- When this is not true, the results of the regression model might be unreliable
- This assumption can be verified by:
  - the “Residual vs. Fitted” plot
  - the Breusch-Pagan Test or the White Test



# The Coefficient of Determination or “R Squared” ( $R^2$ )

- One way to measure how well the least squares regression line “fits” the data is using the **coefficient of determination**, denoted as  $R^2$ .
- $R^2$  is the proportion of the variance in the response variable that can be explained by the predictor variable.
- $R^2$  can range **from 0 to 1**.
  - A **value close to 0** indicates that data is very **spread around the regression line** (this doesn't necessarily mean that the model is a bad fit, rather that the data is naturally noisy)
  - A **value close to 1** indicates that the response variable can be perfectly explained without error by the predictor variable.
- For example, an  $R^2$  of 0.2 indicates that 20% of the variance in the response variable can be explained by the predictor variable; an  $R^2$  of 0.77 indicates that 77% of the variance in the response variable can be explained by the predictor variable
- **BEWARE OF MISINTERPRETATION:** measures variability around a regression line... it **doesn't tell if the model is a good fit or even reasonable !!**
  - To assess the performance of linear models,  $R^2$  must be considered along with other measures (e.g. the Residual Standard Error or the significance level of the regression)

# Multiple Linear Regression

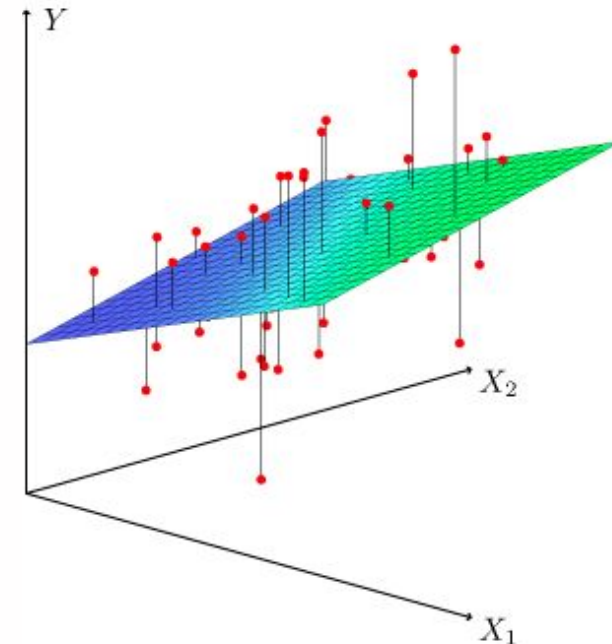
Regression analysis can be used to estimate the linear relationship between a response variable and several predictors

# Multiple linear regression: formally

- A multiple linear regression model takes the form:
- where:
  - : The response variable
  - : The  $j$ th predictor variable
  - : The average effect on of a one unit increase in , holding all other predictors fixed
  - : The error term
- The values for , , ... , are chosen using the least square method, which minimizes the sum of squared residuals (RSS):

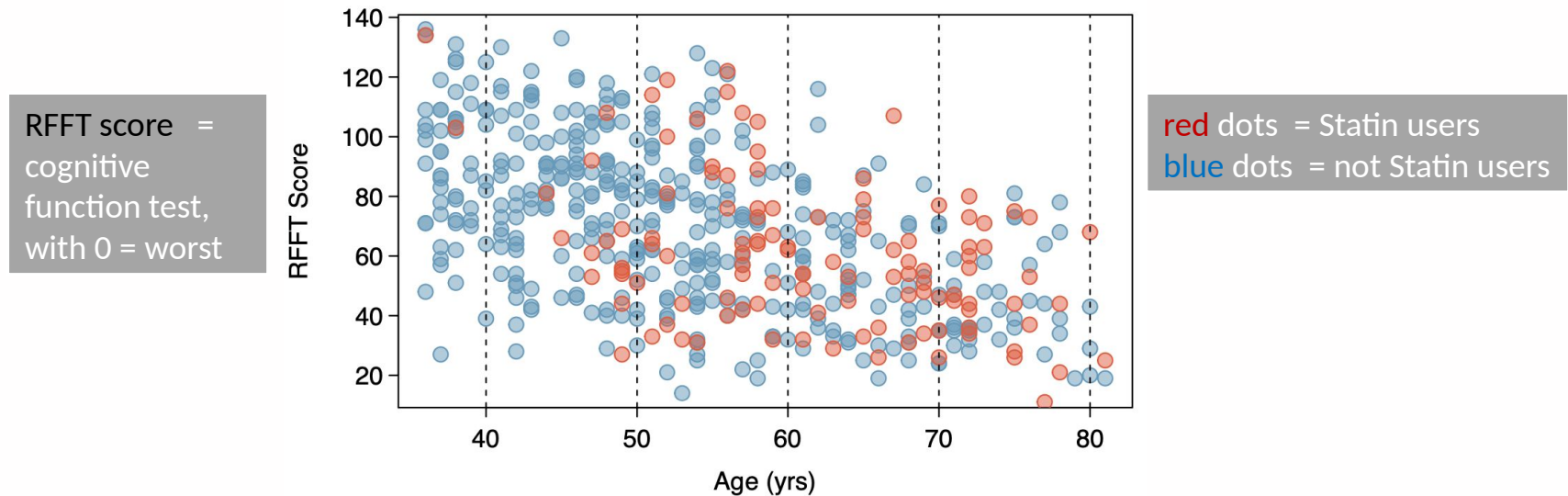
$$RSS =$$

- where:
  - $\sum$ : A greek symbol that means sum
  - : The actual response value for the  $i$ th observation
  - : The predicted response value based on the multiple linear regression model



# Multiple linear regression: example

- [We'll revisit this in the lab, using the PREVEND dataset]
- STUDY: **Statins** are a class of drugs widely used to lower cholesterol (which can increase risk for adverse cardiovascular events). However, treatment with a statin might be associated with an increased risk of **cognitive decline**. Adults of **older age** are at increased risk for cardiovascular disease, but also for cognitive decline
- GOAL: Examine the association of **statin use** with **cognitive ability** in an observational cohort, but also accounted for **age** in the analysis as it could be a potential **confounder** in this setting
- HYPOTHETICAL MODEL:



Source: Vu, J., & Harrington, D. (2021). *Introductory Statistics for the Life and Biomedical Sciences*. Retrieved from <https://www.openintro.org/book/biostat/>

# Multiple linear regression: interpreting predictors coefficients

Given our model, we have obtained this prediction equation:

- ESTIMATE for a coefficient is the predicted mean change in corresponding to a 1 unit change in , when the values of all other predictors remain constant. E.g.:
  - an increase of 1 year of age is associated with a decrease of -1.2710 in RFFT score, when statin use is the same
  - for 2 individuals of the same age, the RFFT score will be 0.8509 higher for the one taking statins
- [STD. ERROR, T-STATISTIC, P-VALUE]: For each coefficient the model tests the  $H_0 : = 0$ 
  - the association between RFFT score and statin use is not statistically significant, but the association between RFFT score and age is significant

```
lm(formula = rfft ~ statin + age, data = prevend)
```

```
Residuals:
```

Min	1Q	Median	3Q	Max
-63.855	-16.860	-1.178	15.730	58.751

```
Coefficients:
```

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	137.8822	5.1221	26.919	<2e-16 ***
statin	0.8509	2.5957	0.328	0.743
age	-1.2710	0.0943	-13.478	<2e-16 ***

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```



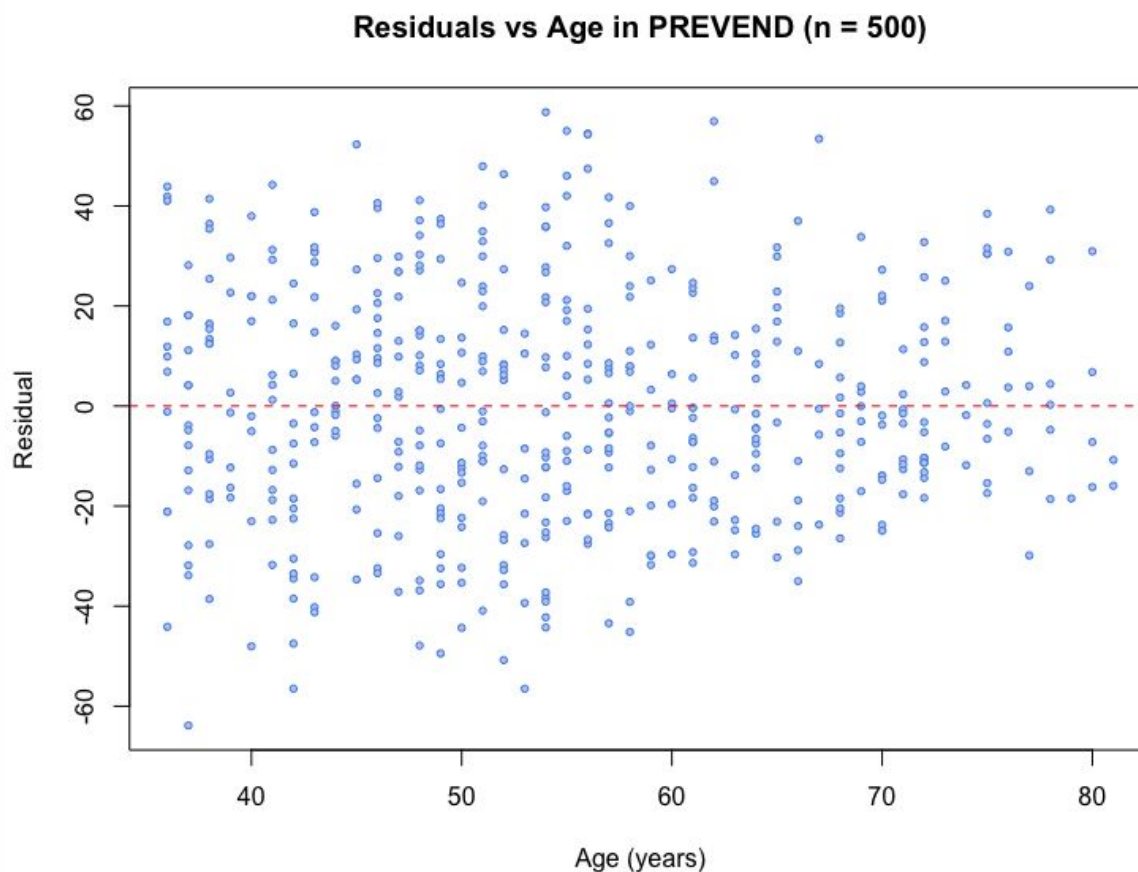
# Assumptions for (multiple) linear regression

Similar to those of simple linear regression...

1. **Linearity** : For each predictor variable , change in the predictor is linearly related to change in the response variable when the value of all other predictors is held constant.
  - It is not possible to make a scatterplot of a response against several simultaneous predictors. Instead, we use a **modified residual plot** to assess linearity
2. **Normality of residuals**: The residuals are approximately normally distributed.
  - Verified with normal probability plots (Q-Q plots etc.)
3. **Homoscedasticity** (constant variability): The residuals have approximately constant variance at every level of x.
  - Verified by plotting the residual values on the **y-axis** and the predicted values on the **x-axis**
4. **Independent observations**: Each set of observations ( ) is independent
5. **(NEW!) No multicollinearity**: i.e. no situations when there is a strong linear correlation between the independent variables, conditional on the other variables in the model
  - multicollinearity may lead to imprecision or instability of the estimated parameters when a variable changes

## Using residuals to check model assumption 1 (on individual predictors)

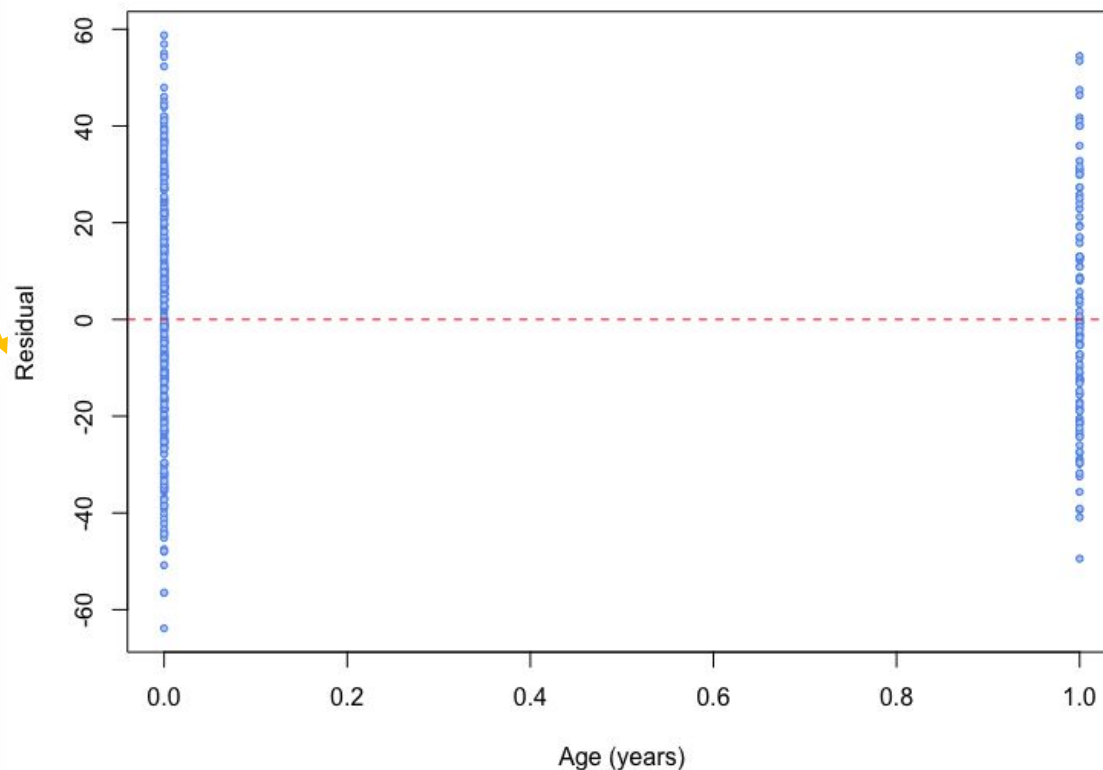
- Assess **linearity** with respect to **age** using a scatterplot with **residual values** on the **y-axis** and values of **age** on the **x-axis**
  - There does not seem to be remaining nonlinearity with respect to **age** after the model is fit.



## Using residuals to check model assumption 1 (on individual predictors)

- Assess **linearity** with respect to **statin use** using a scatterplot with **residual values** on the **y-axis** and values of age on the **x-axis**
  - It is not necessary to assess linearity with respect to **statin use** since it is measured as a **categorical variable**. A line drawn through two points (that is, the mean of the two groups defined by a binary variable) is necessarily linear

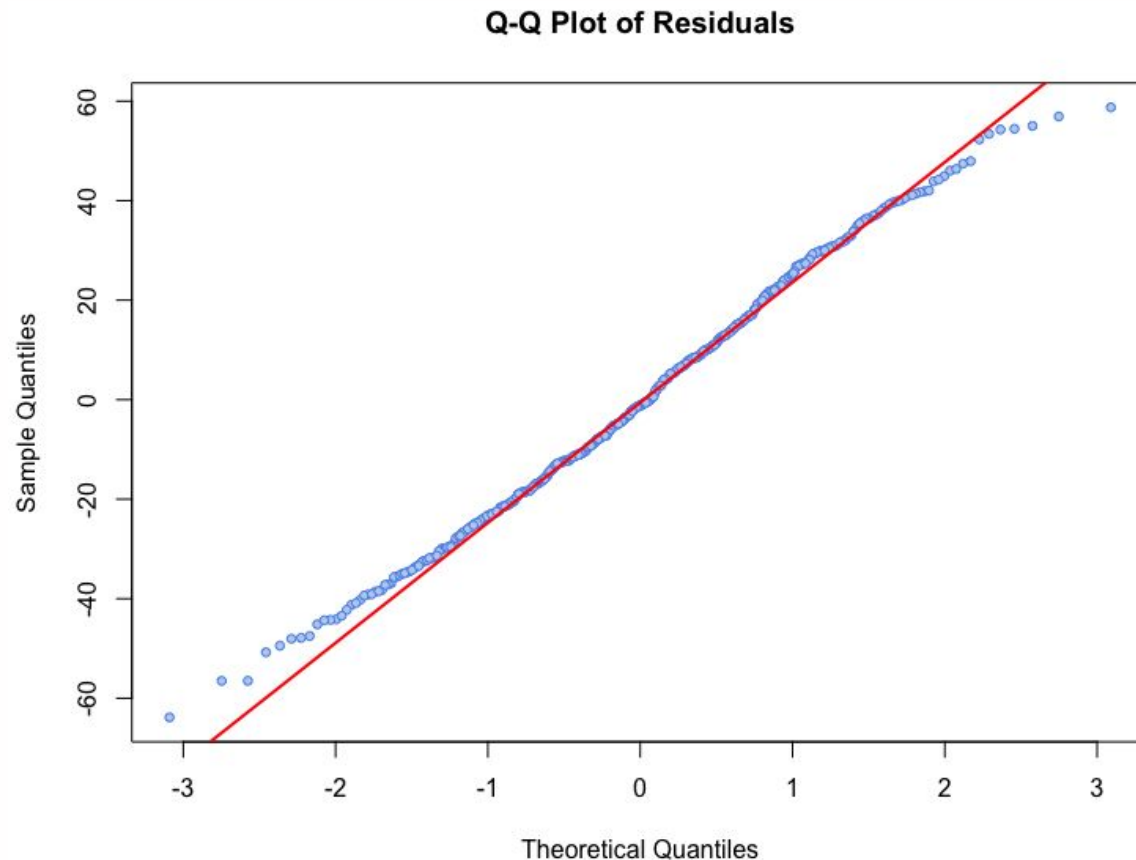
Residuals vs Age in PREVEND (n = 500)



NOT  
MEANINGFUL  
with respect  
to categorical  
explanatory  
variable!

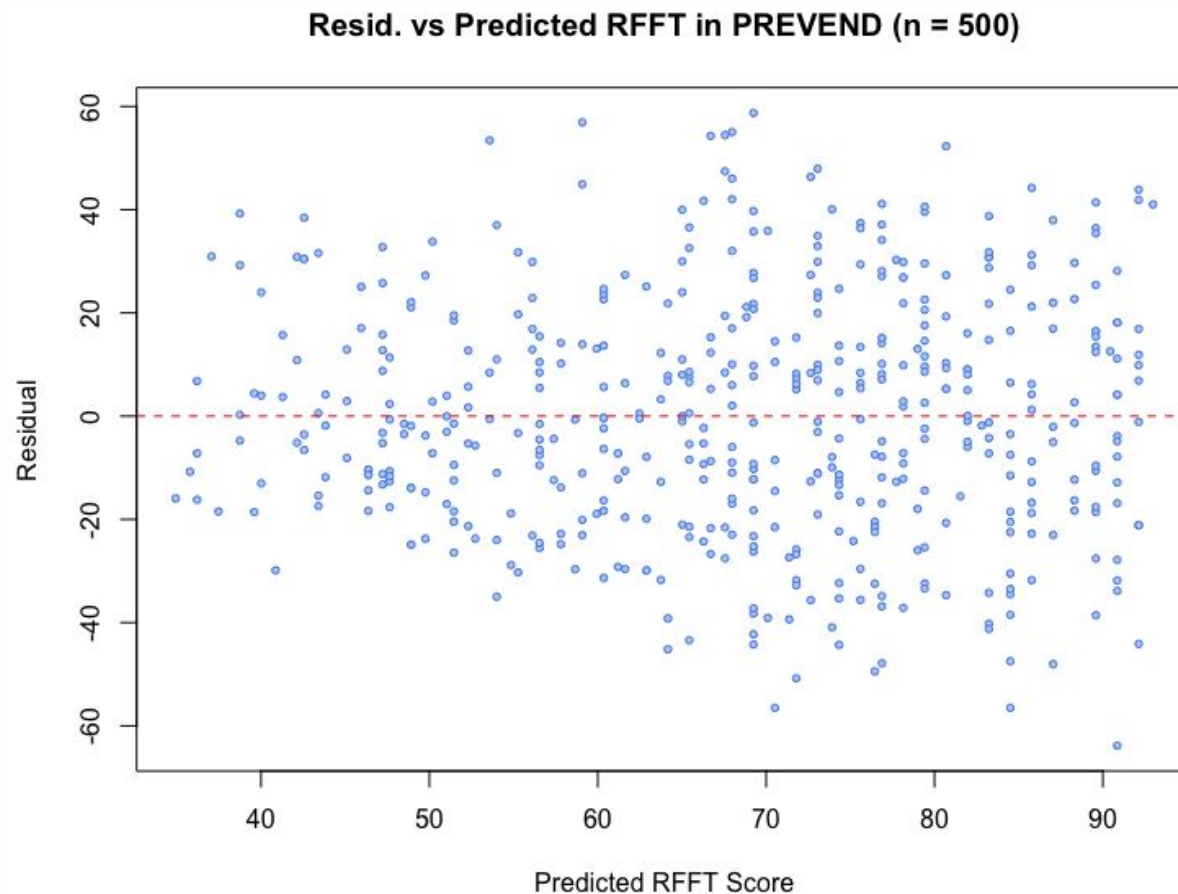
## Using residuals to check model assumption 2 (Normality of residuals)

- As in Simple Regression we use Q-Q plots
  - The residuals are reasonably normally distributed, with only slight departures from normality in the tails.



# Using residuals to check model assumption 3 (Homoscedasticity)

- As in Simple Regression we plot the residual values on the **y-axis** and the predicted values on the **x-axis**
  - It seems reasonable to assume approximately constant variance.



# Checking assumption n 6 (no multicollinearity)

- It can be assessed by studying the correlation between each pair of independent variables, or even better, by computing the **variance inflation factor (VIF)**
  - The **VIF** measures how much the variance of an estimated regression coefficient increases, relative to a situation in which the explanatory variables are strictly independent.
  - A **high value of VIF is a sign of multicollinearity** (the threshold is generally at 5 or 10)
  - The easiest way to reduce the VIF is to remove some correlated independent variables, or eventually to standardize the data.

## Collinearity

High collinearity (VIF) may inflate parameter uncertainty



Not an  
issue in our  
dataset/  
model

# Variability in the response explained by the model: $R^2$ and Adj. $R^2$ in multiple regression

- As in simple regression, represents the proportion of variability in the response variable explained by the model
  - As variables are added, always increases
- incorporates a *penalty* for including predictors that do not contribute much towards explaining observed variation in the response variable
  - does not have an inherent interpretation, but it is useful while comparing models with different explanatory variables
- **Resid. Std. Err.** (square root of the residual mean squared errors ) is the estimated standard deviation of the error of the regression equation and is a good measure of the accuracy of the regression line.

```
Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept) 137.8822    5.1221   26.919  <2e-16 ***
statin       0.8509     2.5957    0.328    0.743
age         -1.2710     0.0943  -13.478  <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 23.21 on 497 degrees of freedom
Multiple R-squared:  0.2852,    Adjusted R-squared:  0.2823
F-statistic: 99.13 on 2 and 497 DF,  p-value: < 2.2e-16
```

# F statistic in multiple regression

- Again, the **F-test of overall significance** indicates whether this linear regression model provides a **better fit to the data** than a hypothetical model that contains no independent variables (known as the “**intercept model**”)
  - $H_0$ : (null hypothesis) The **intercept model** fits the data as well as your model.
  - $H_1$ : (alternative hypothesis) Your model fits the data better than the intercept-only model
- In this case **p-value** is extremely small, we have sufficient evidence to conclude that this model fits the data better than intercept-only model
- NOTE: in general, if none of the independent variables are statistically significant, the overall F-test is also not statistically significant

```
Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept) 137.8822    5.1221   26.919  <2e-16 ***
statin       0.8509     2.5957    0.328    0.743
age        -1.2710     0.0943  -13.478  <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 23.21 on 497 degrees of freedom
Multiple R-squared:  0.2852,    Adjusted R-squared:  0.2823
F-statistic: 99.13 on 2 and 497 DF,  p-value: < 2.2e-16
```



# Model performance: ideas for further investigation

- The **PREVEND** data came from a **cross-sectional study**, i.e. not from a study in which participants were followed as they aged (i.e., a **longitudinal study**)
- So, while the model indicates that older patients tend to have lower RFFT scores, **we cannot conclude that RFFT scores decline with age in individuals**
  - only **repeated measurements** of RFFT taken as (the same) individual participants aged could rule out some explanatory effect of unobserved differences across different age cohorts
- We found that **age** was a *confounder*: **was it the only one?**
  - Other **potential confounders** could be **education level** (also associated to access to health care) and the **presence of cardiovascular disease** (can lead to vascular dementia and cognitive decline)
  - **Residual confounders** —frequent in observational studies— can be other variables in a dataset that have not been examined, or variables that were not measured in the study
- A **randomized experiment** is the best way to eliminate **residual confounders**, since it ensures that, at least on average, all predictors are not associated with the exposure (i.e. one source of confounding: **selection bias**).

*The details of how a study was designed and how data were collected should always be taken into account when interpreting study results.*

# Adding a categorical predictor with several levels to the model

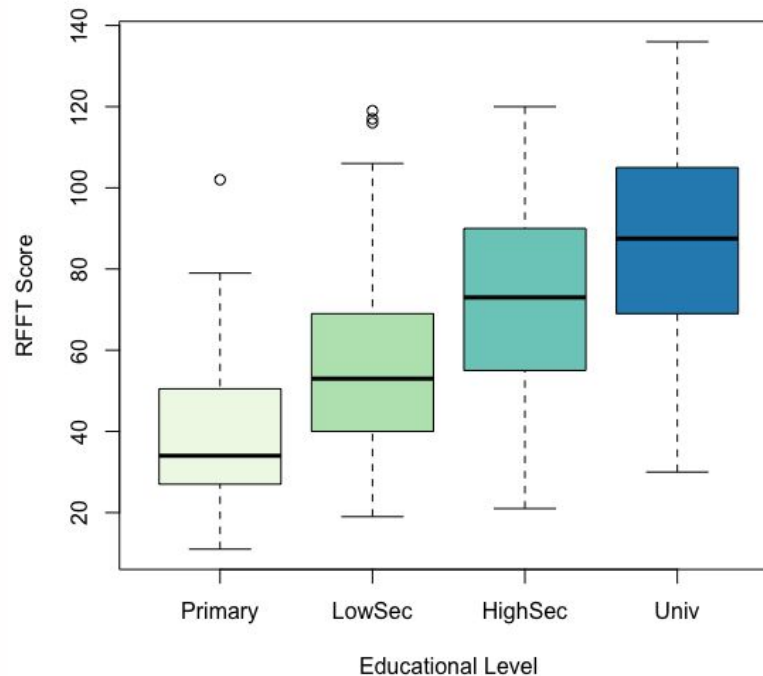
- In a regression model with a **categorical variable with more than two levels** (e.g. education level), **one of the categories is set as the reference category**. The remaining categories each have an estimated coefficient
  - Each predictor levels can be thought of as binary variables that can take on either 0 or 1

- **EXAMPLE:** predicted RFFT for individuals in **Lower Secondary Education** level

= 55.72

is not in the model because it is the **implicit reference level** (i.e. the intercept value 40.94)

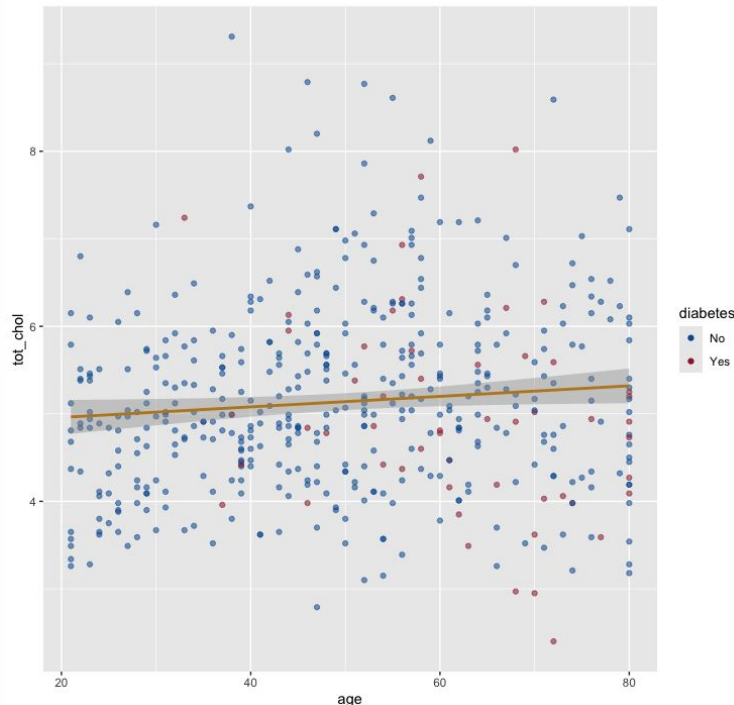
RFFT by Education in PREVEND (n = 500)



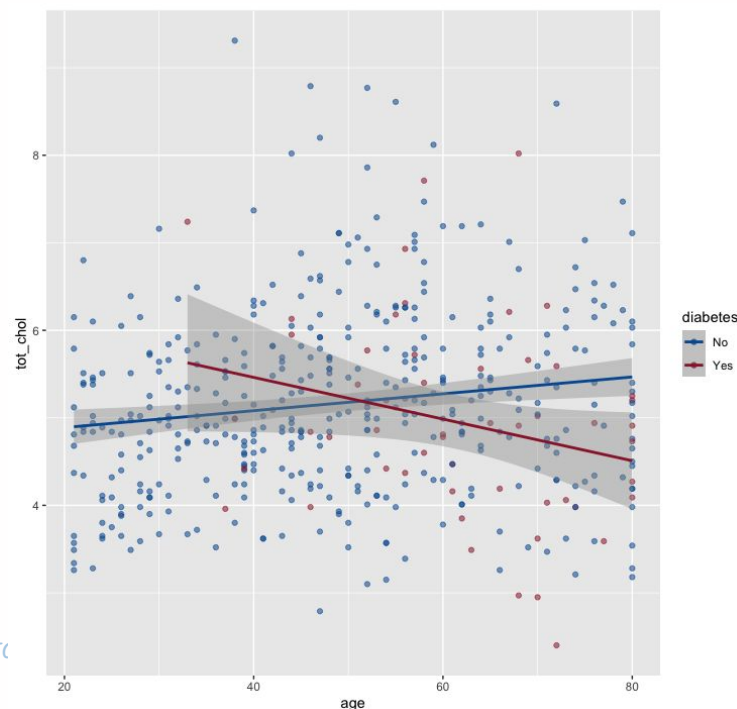
# Adding a interaction term to the model specification

- A statistical interaction occurs when the effect of one explanatory variable  $X_1$  on the response  $Y$  depends on the level of another explanatory variable  $X_2$
- Let's go back to the NHANES dataset and consider a linear model that predicts total cholesterol level (mmol/L) from age (yrs.) and diabetes status.
- Comparing 2 alternative models:
  1. MLR without interaction:
  2. MLR with interaction:  $+\beta_2(\text{Diabetes} \times \text{Age})$
- Model 2 acknowledges the relationship between cholesterol and age depends on diabetes status (i.e. it “allows” the relationship of with the to vary based on the values of)

Linear model on entire sample



Linear model by category (Diabetics or not)



# Multiple linear regression: recap

- **Multiple linear regression** is a generalization of simple linear regression to address the relationship between a **response variable** and **several predictors**, where  $k$  is the number of predictors
  - including logical, interval/ratio, or categorical predictors, as well as interaction terms
  - to interpret categorical predictors (>2 levels) one of the category's levels is set as the reference, each remaining level has an estimated coefficient = estimated change relative to the reference
- **Typical applications** of multiple linear regression are:
  1. **PRIMARY PREDICTOR:** Estimating an association between a response variable and **primary predictor** of interest, while adjusting for **possible confounding variables**
    - this is the case of the previous example! (Examining the association between **statin use** and **cognitive ability**, adjusting by **age**)
  2. **EXPLANATORY MODELS:** Constructing a model that effectively explains the observed variation in the response variable; in other words, to **build a predictive model for a response variable**
    - different techniques may be adopted in model selection (i.e. different **specifications** where we add/subtract explanatory variables)
    - A **parsimonious model (few variables)** is usually preferred over a complex model
    - **$R^2$**  and **Adjusted  $R^2$**  can be useful to compare models
    - In particular **Adjusted  $R^2$**  helps to balance predictive ability with complexity in a multiple regression model

# More advanced topics on REGRESSION...

- This lecture is just an introduction but there is a wide array of topics pertaining to regression analysis...
- Here are some of the many variants and advancements over the linear regression model:
  - **LOGISTIC REGRESSION**: if the dependent variable is dichotomous (0,1) or nominally scaled
  - **POISSON REGRESSION**: if the dependent variable is count over a period of time
  - **COX PROPORTIONAL HAZARDS REGRESSION**: for modeling censored data
  - **FUNCTIONAL TRANSFORMATIONS**: quadratic, exponential ....
  - **GENERALIZED LINEAR MODELS (GLMs)**: an extension of the linear model where the modelling of error is not Gaussian
  - **PANEL REGRESSION MODELS**: special regression models that can make use of both the temporal and the inter-individual variation if you have longitudinal data (or time-series cross-sectional or panel data)

# Shifting emphasis on empirical outcome prediction

Introduction to Machine Learning (ML)  
models

# A conceptual framework to understand different types of statistical **modeling** (part 2/2)

1. **association/correlation** → observational studies
2. **causal explanation** → experimental studies
3. **empirical prediction** → algorithmic machine learning and data-mining modeling
  - aimed at **predicting new or future observations** (without necessarily explaining how)
  - relies on **big data**
  - prevalent in fields like natural language processing, **bioinformatics**, etc.. In **epidemiology**, there is more of a mix causal explanation & empirical prediction

- **NOTES:**

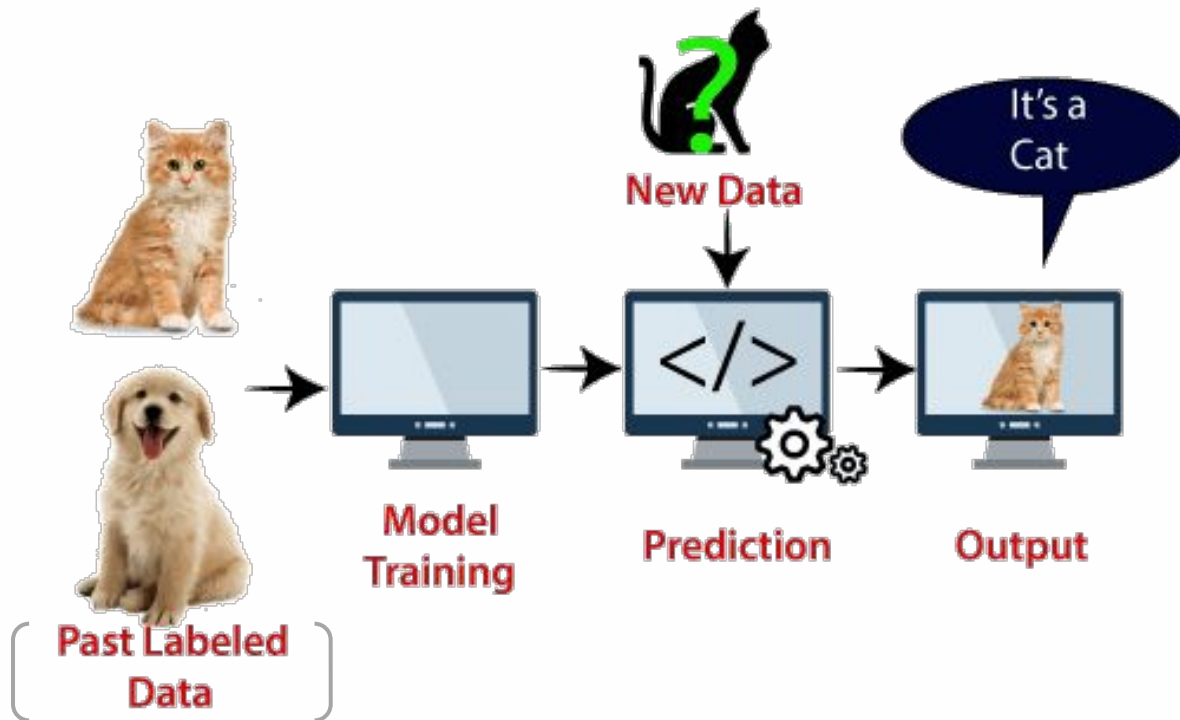
- ✓ “Prediction” does not necessarily refer to future events, but rather to *future* datasets that were previously unseen to the algorithm

# Defining Machine Learning (ML)



*“At its core, **Machine Learning** is just a “thing-labeler”, taking something and telling you what label It should get.”*

*(Cassie Kozyrkov)*



Source: Image from <https://entri.app/blog/what-is-svm-algorithm-in-machine-learning/>



# Defining Machine Learning (ML)

- **Machine Learning** is a broad and highly active research field. (In the life sciences, “*precision medicine*” is an application of machine learning to biomedical data)
- The **general idea** is to **predict** or **discover outcomes from measured predictors**, in problems like:
  - *Can we discover new types of cancer from gene expression profiles?*
  - *Can we predict drug response from a series of genotypes?*
  - *How do we classify a set of images/spectrometry outputs, etc.*
  - *Given various clinical parameters, how can we use them to predict heart attacks?*
- The **ML is a data-driven (inductive) approach**, where a machine *\*learns\** the rules/patterns from a set of **training data** and (then) *\*validates\** findings on a set of **testing data**
- In contrast with inferential statistics, **ML doesn't worry about assumptions on parameters** (probability distribution, error, correlation, etc.), **nor the causal nexus** between specific predictor(s) and response, **nor the data collection strategy**
- In contrast with standard statistics, **in ML the rules are not necessarily specified...** hence ML = a subfield of AI

# Stylized comparison between statistics and machine-learning

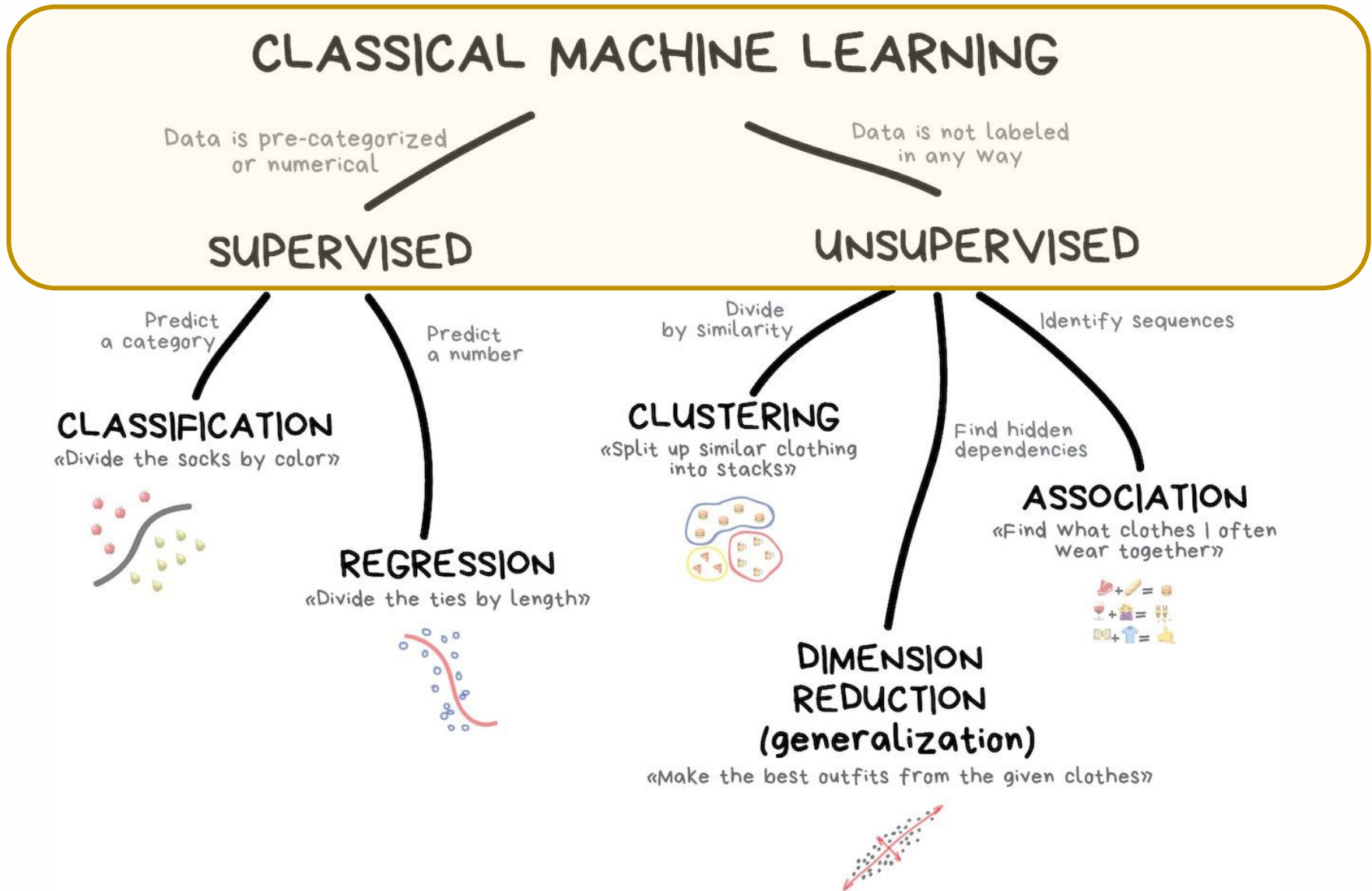
	Standard (causal inference) Statistics	Machine Learning
<b>Typical Goal</b>	Explanation, uncovering causal relationships	Predicting an outcome as accurately as possible
<b>Typical Task</b>	Research based on a theory to identify the <u>causal effect</u> (better: pre-register your hypothesized model).	Try out and tune many different algorithms in order to <u>maximize predictive accuracy</u> in new and unseen test datasets.
<b>Data generating process</b>	Designed ex-ante based on study goal (e.g. randomized control trial, or observational study with statistical control variables)	Useful but not strictly necessary, and often not available
<b>Parameters of interest:</b>	Causal effect size and statistical significance, p-value of <u>treatment X</u> for outcome Y	Model's accuracy (%), precision/recall, sensitivity/specificity, in <u>predicting Y</u>
<b>Dataset</b>	Use <b>ALL AVAILABLE DATA</b> to calculate effect of interest (it was designed to be representative of a population).	It is critical to <b>SPLIT THE DATA</b> (usually 75% for training and 25% for testing the algorithms) leaving aside a sub-sample to test the model with unseen new data

Source: Adapted from <https://forloopsandpiekicks.wordpress.com/2022/02/10/beginners-guide-to-machine-learning-in-r-with-step-by-step-tutorial/>

# Supervised or Unsupervised ML algorithms?

....another conceptual framework

# A fundamental distinction: supervised and unsupervised ML



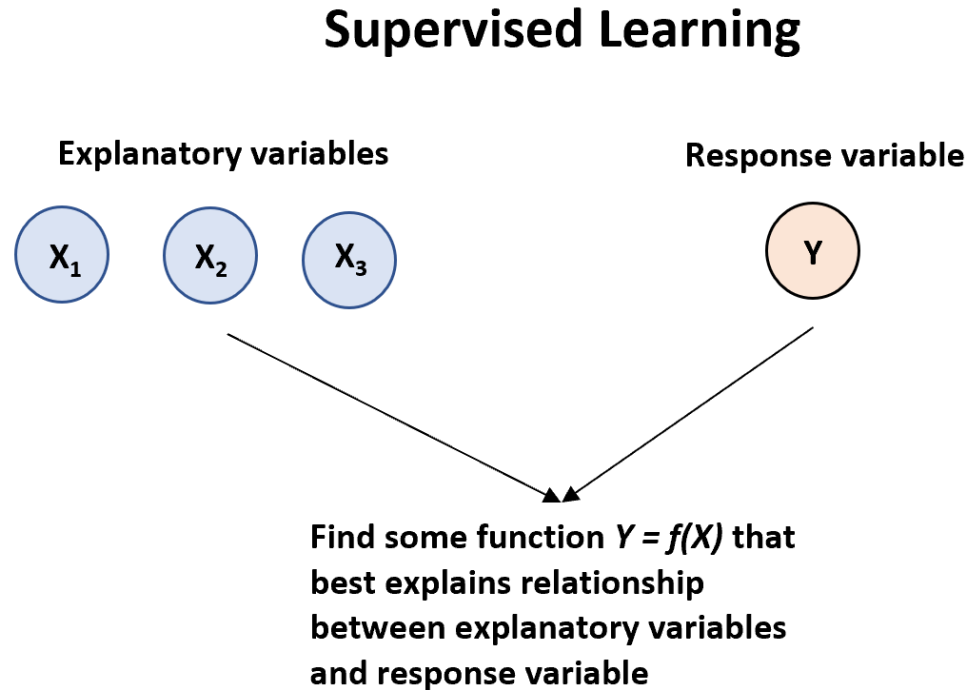
Source: Image from [https://vas3k.com/blog/machine\\_learning/index.html](https://vas3k.com/blog/machine_learning/index.html)

# A fundamental distinction: supervised and unsupervised ML

- ML includes many different algorithms that can be used for understanding data. These algorithms can be classified as:
  - **Supervised Learning Algorithms:**
    - building a model to estimate or predict an output based on one or more inputs
      - **Regression:** Modeling a relationship, the typical output variable is continuous (e.g. weight, height, time, etc.) or dichotomous.
      - **Classification:** Splits objects based on one of the attributes known beforehand. The the typical output variable is categorical (e.g. male or female, pass or fail, benign or malignant, etc.)
  - **Unsupervised Learning Algorithms:**
    - finding structure and relationships among inputs. There is no “supervising” output
      - **Clustering:** Finding “clusters” of observations in a dataset that are similar to each other (*based on unknown features*).
      - **Association:** Finding “rules” that can be used to draw associations. For example, if a patient has a high biomarker X, he will have a low biomarker Y.
      - **Dimension reduction:** Assembling specific features into more high-level ones (e.g. PCA)

# Supervised ML algorithms

- A supervised learning algorithm can be used when we have **one or more explanatory variables** ( $X_1, X_2, X_3, \dots, X_p$ ) and a **response variable** ( $Y$ ) and we would like to find some function that describes the relationship between the explanatory variables and the response variable:
- $Y = f(X) + \epsilon$
- where
  - $f()$  represents **systematic information that X provides about Y** and where
  - $\epsilon$  is a random error term independent of  $X$  with a mean of zero.



Source: <https://www.statology.org/supervised-vs-unsupervised-learning/>

# Supervised Learning Algorithms **purpose**

There are two main reasons to use supervised learning algorithms:

1. **Prediction:** We often use a set of explanatory variables to predict the value of some response variable (e.g. using square footage and number of bedrooms to predict home price)
  2. **Inference:** We may be interested in understanding the way that a response variable is affected as the value of the explanatory variables change (e.g. how much does home price increase, on average, when the number of bedrooms increases by one?)
- *Depending on whether our goal is inference or prediction (or a mix of both), we may use different methods for estimating the function  $f$ . For example, linear models offer easier interpretation but non-linear models that are difficult to interpret may offer more accurate prediction.*



# Supervised Learning: commonly used algorithms

Most commonly used supervised learning algorithms:

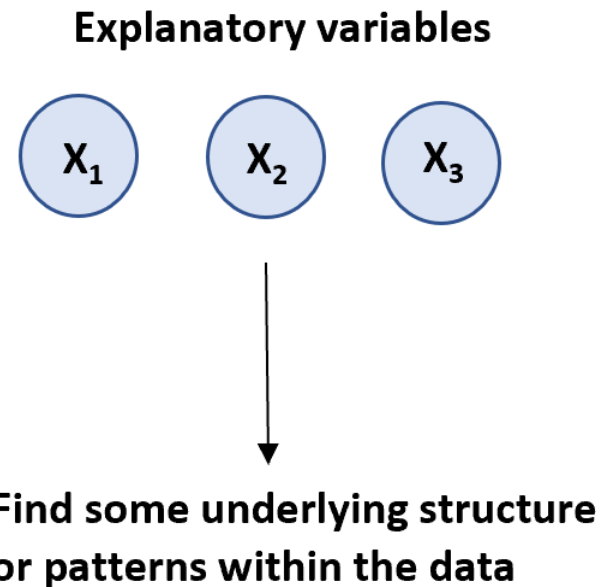
- Linear regression
- Logistic regression
- Linear discriminant analysis
- Quadratic discriminant analysis
- Decision trees
- Naive bayes
- Support vector machines
- Neural networks

# Unsupervised ML algorithms

Example of PCA

An unsupervised learning algorithm can be used when we have a list of variables ( $X_1, X_2, X_3, \dots, X_p$ ) and we would simply like to find underlying structure or patterns within the data.

## Unsupervised Learning



Source: <https://www.statology.org/supervised-vs-unsupervised-learning/>

## Supervised Learning Algorithms typical purpose

There are two main types of unsupervised learning algorithms:

1. **Clustering:** Using these types of algorithms, we attempt to find “clusters” of observations in a dataset that are similar to each other. This is often used in retail when a company would like to identify clusters of customers who have similar shopping habits so that they can create specific marketing strategies that target certain clusters of customers.
  2. **Association:** Using these types of algorithms, we attempt to find “rules” that can be used to draw associations. For example, retailers may develop an association algorithm that says “if a customer buys product X they are highly likely to also buy product Y.”
- Most commonly used unsupervised learning algorithms:
    - Principal component analysis
    - K-means clustering
    - K-medoids clustering
    - Hierarchical clustering
    - Apriori algorithm

# Summary: Supervised vs. Unsupervised Learning

- Here are the key differences between supervised and unsupervised learning algorithms:

	Supervised Learning	Unsupervised Learning
<b>Description</b>	Involves building a model to estimate or predict an output based on one or more inputs.	Involves finding structure and relationships from inputs. There is no “supervising” output.
<b>Variables</b>	Explanatory and Response variables	Explanatory variables only
<b>End goal</b>	Develop model to <b>(1)</b> predict new values or <b>(2)</b> understand existing relationship between explanatory and response variables	Develop model to <b>(1)</b> place observations from a dataset into a specific cluster or to <b>(2)</b> create rules to identify associations between variables.
<b>Types of algorithms</b>	<b>(1)</b> Regression and <b>(2)</b> Classification	<b>(1)</b> Clustering and <b>(2)</b> Association

Source: <https://www.statology.org/supervised-vs-unsupervised-learning/>