



One Way ANOVA

One Way ANOVA (**A**nalysis of **V**ariance)

Elias Zintzaras, M.Sc., Ph.D.

*Professor in Biomathematics-Biometry
Department of Biomathematics
School of Medicine
University of Thessaly*

*Institute for Clinical Research and Health Policy Studies
Tufts University School of Medicine
Boston, MA, USA*

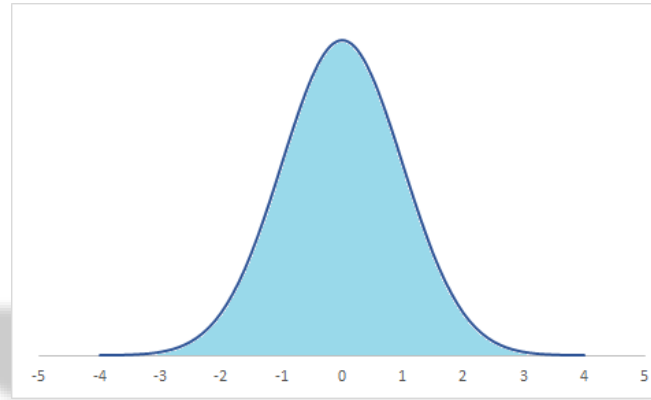
*Theodoros Mprotsis, MSc, PhD
Teacher & Research Fellow
(<http://biomath.med.uth.gr>)
University of Thessaly
Email: tmprotsis@uth.gr*



Why ANOVA;

- Up to this point, we have been comparing two populations
 - Independent samples t-test
 - Paired samples t-test
- Of course limiting ourselves to the comparison of two populations is well ... limiting
- What if we wish to compare the means of more than two populations?
- What if we wish to compare populations each containing several subgroups?
- For this reasons, we will make use of ANOVA (**A**nalysis **o**f **V**ariance)

Suppose we want to compare three sample means to see if there are differences between them

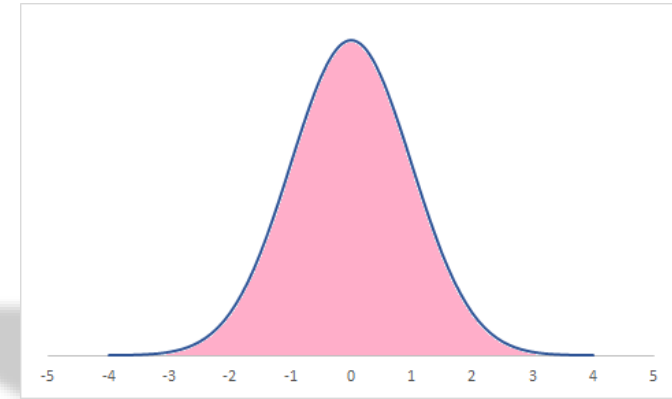


\bar{x}_1

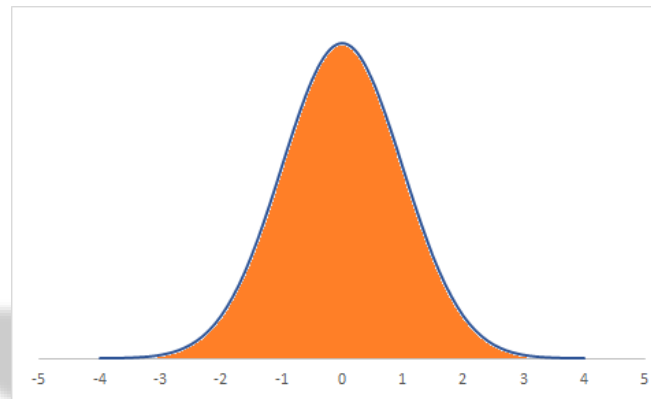
Is one mean so far away from the other two that is likely not from the same population?

What we are asking is:

Do all these three means come from the same population?

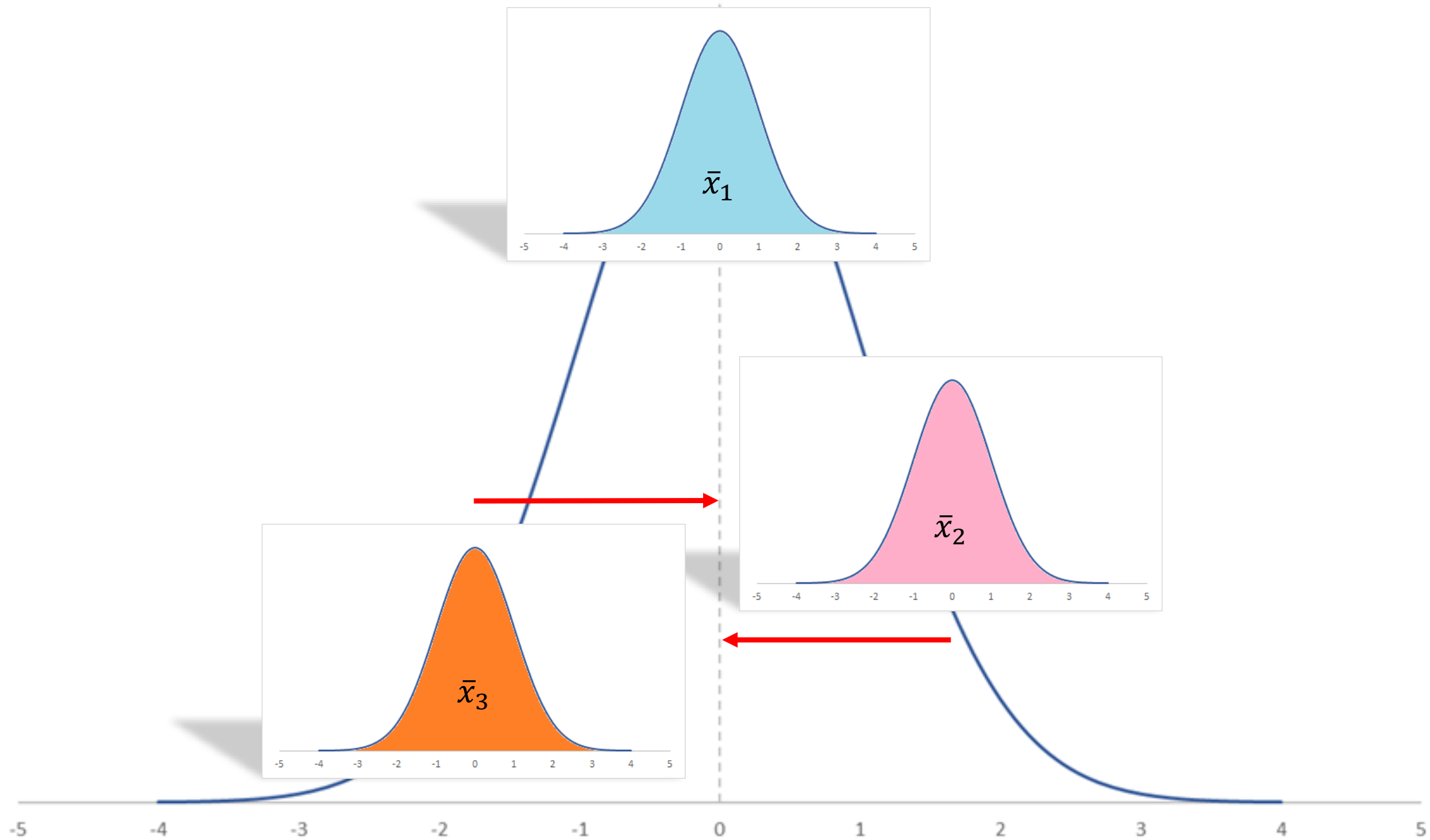


\bar{x}_2

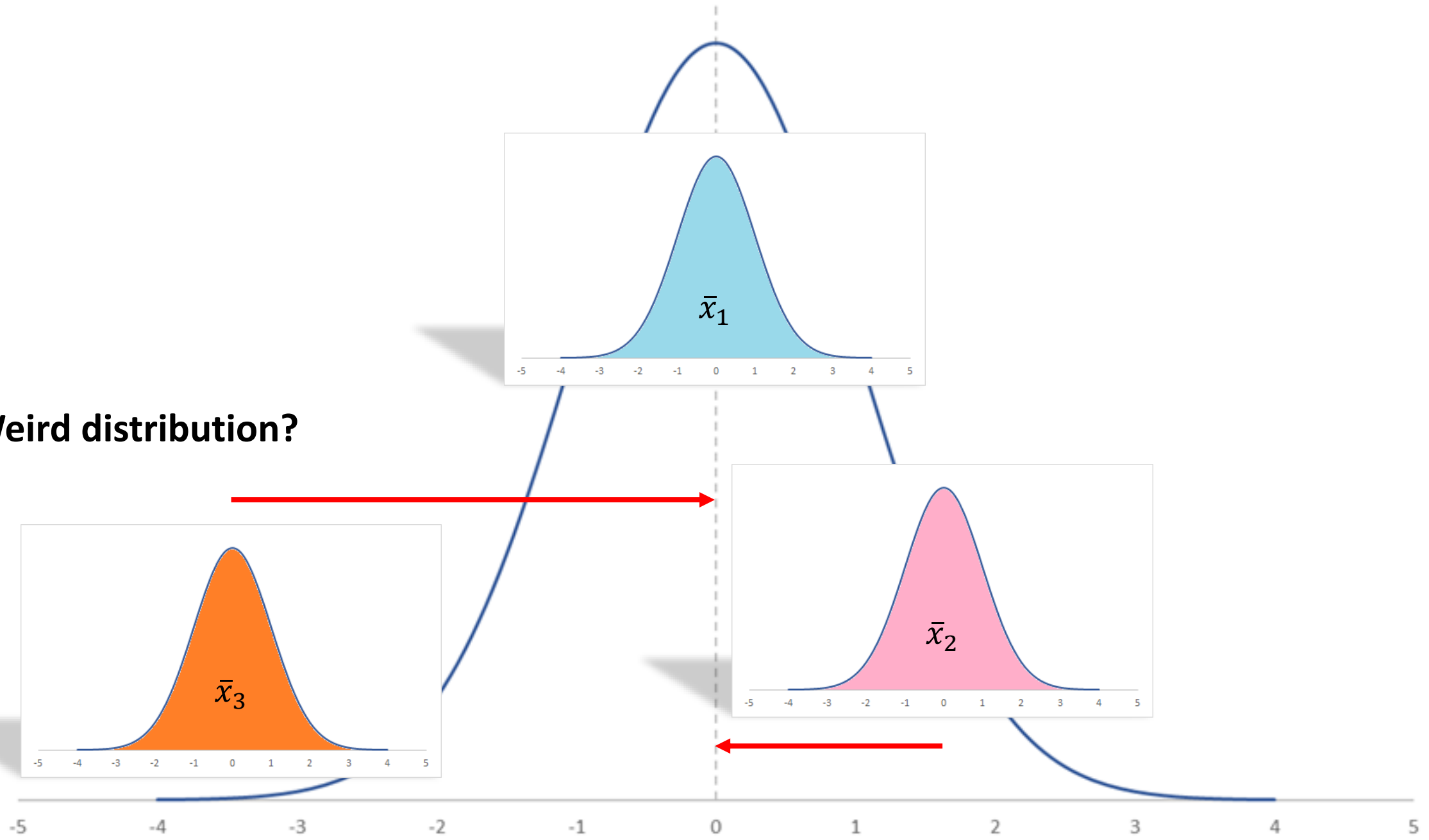


\bar{x}_3

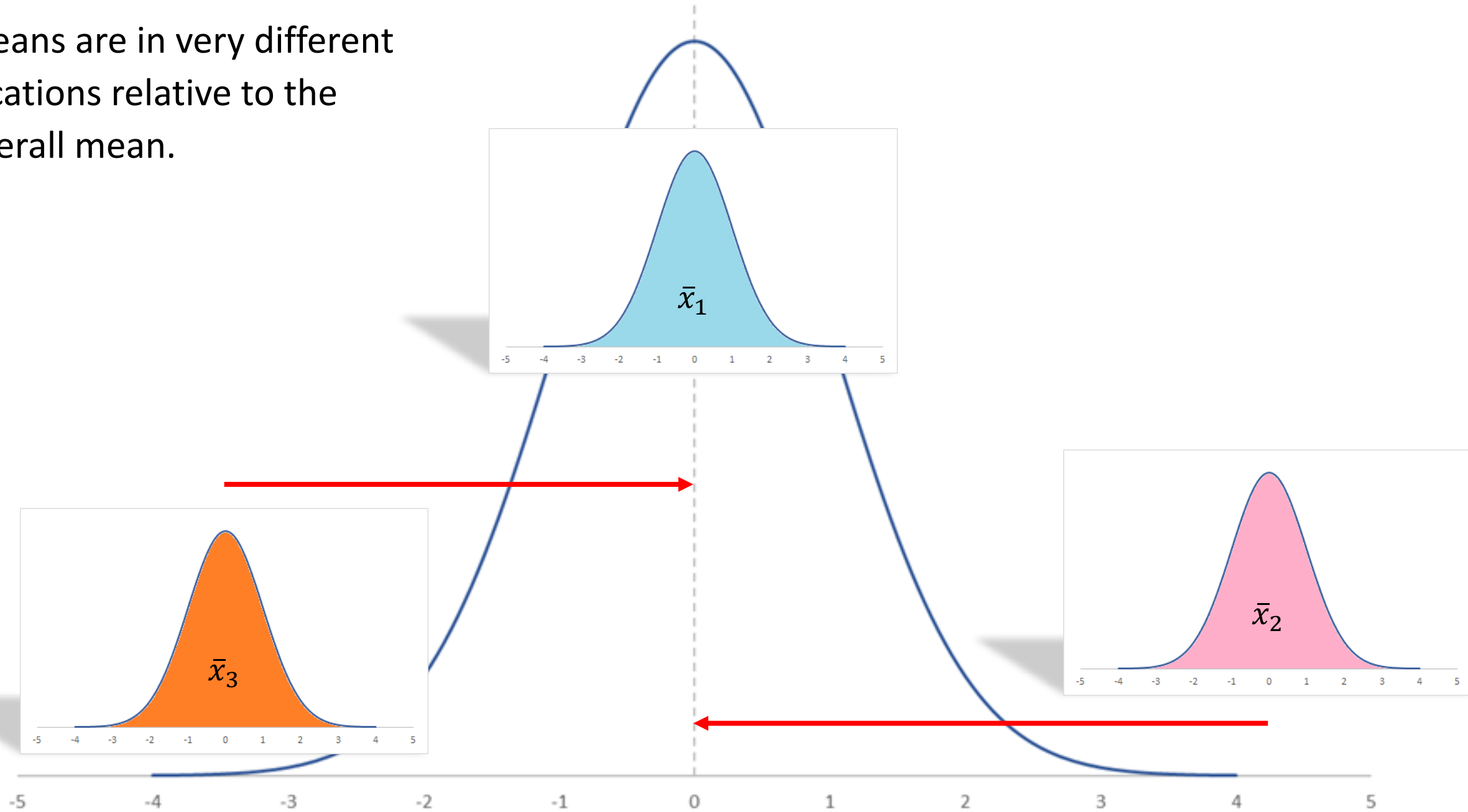
Or are all three so far apart that they ALL likely come from different populations?



Weird distribution?



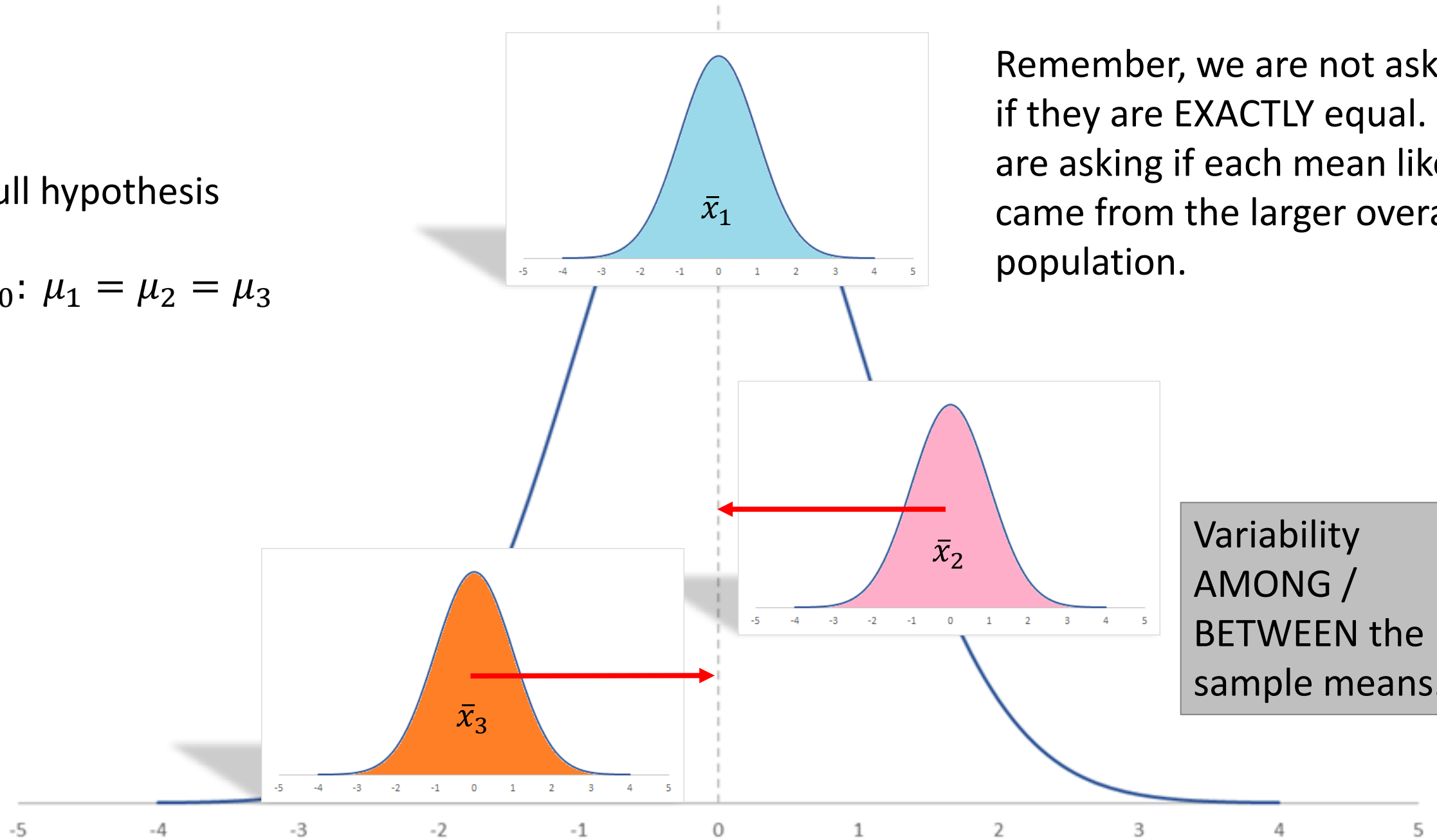
Means are in very different locations relative to the overall mean.



Null hypothesis

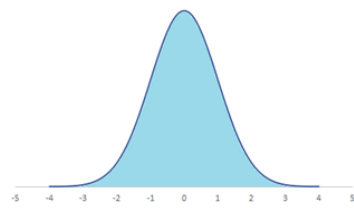
$$H_0: \mu_1 = \mu_2 = \mu_3$$

Remember, we are not asking if they are EXACTLY equal. We are asking if each mean likely came from the larger overall population.

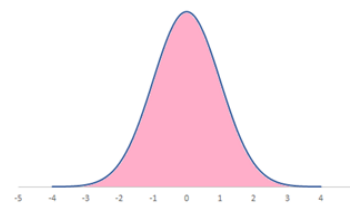


Variability
AMONG /
BETWEEN the
sample means.

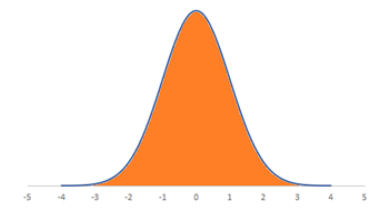
Multiple t-tests



\bar{x}_1



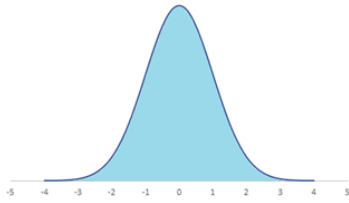
\bar{x}_2



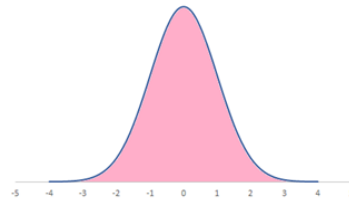
\bar{x}_3

$$H_0: \bar{x}_1 = \bar{x}_2; \alpha = .05$$

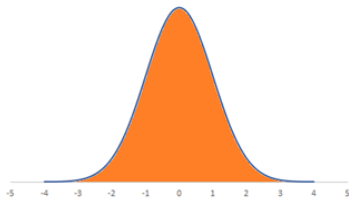
$$H_0: \bar{x}_1 = \bar{x}_3; \alpha = .05$$



\bar{x}_1



\bar{x}_2



\bar{x}_3

Pairwise comparisons means three t-tests, ALL with $\alpha = .05$. Type I error rate at 95% confidence.

BUT error COMPOUNDS with each t-test:
 $(1 - .05)(1 - .05)(1 - .05) = 0.857$

$$H_0: \bar{x}_2 = \bar{x}_3; \alpha = .05$$

$$\alpha = 1 - .857 = .143$$

Σφάλμα τύπου I: παρουσιάζεται όταν ο ερευνητής απορρίπτει εσφαλμένα τη μηδενική υπόθεση

What changed?

The variance of each distribution.

Variability
WITHIN the
distributions.

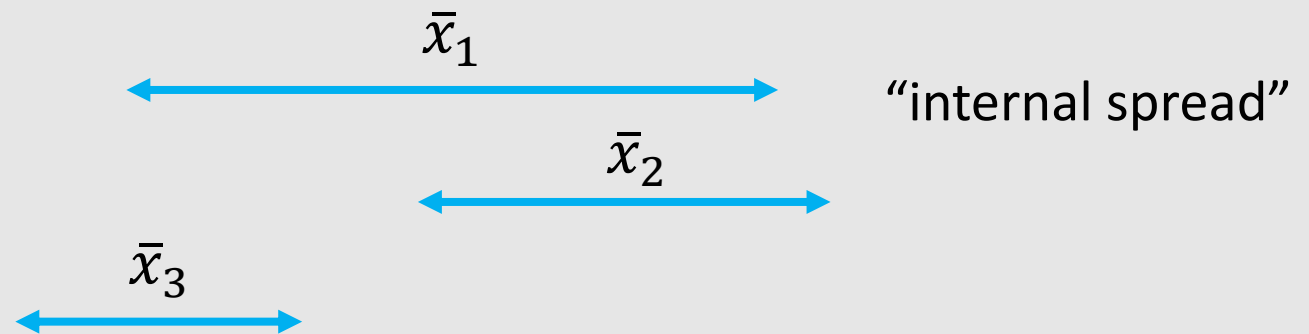
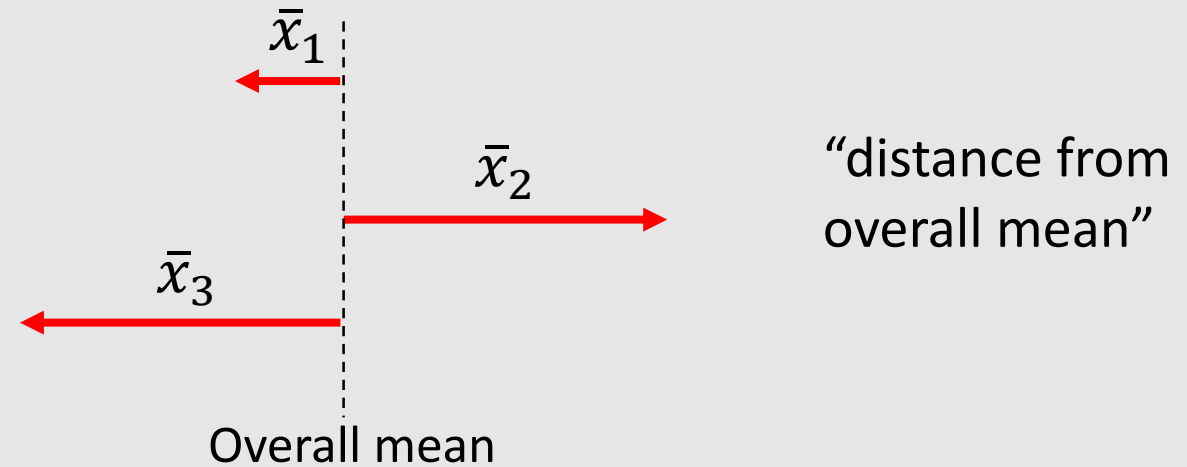




ANOVA: Analysis of Variance is a variability ratio

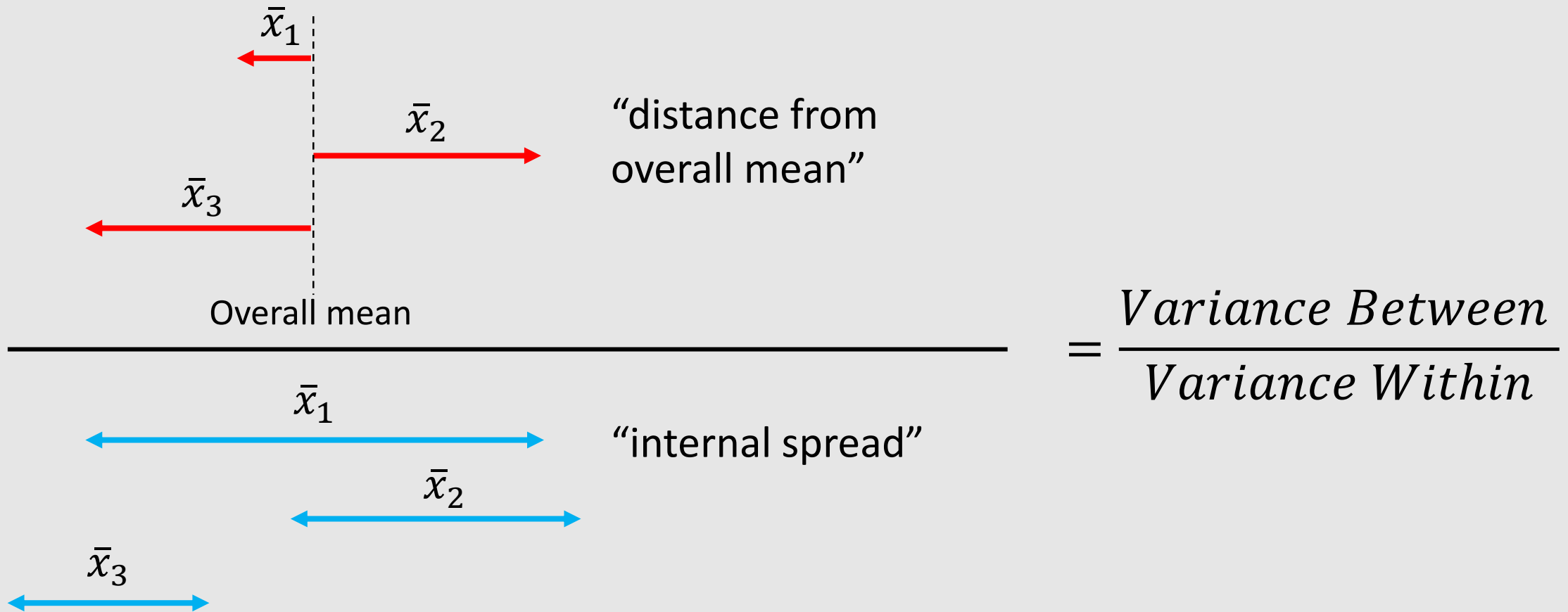
Variability
AMONG / BETWEEN
the sample means.

Variability
WITHIN the
distributions.





ANOVA: Analysis of Variance is a variability ratio





ANOVA: Analysis of Variance is a variability ratio

$$\frac{\textit{Variance Between}}{\textit{Variance Within}} \left. \vphantom{\frac{\textit{Variance Between}}{\textit{Variance Within}}} \right\} \textit{Total Variance Components}$$

$$\textit{Variance Between} + \textit{Variance Within} = \textit{Total Variance}$$

Partitioning – separating total variance into its component parts

If the variability **BETWEEN** the means (distance from the overall mean) in the numerator is relatively large compared to the variance **WITHIN** the samples (internal spread) in the denominator, the ratio will be much larger than 1. The samples then most likely do **NOT** come from a common population; **REJECT NULL HYPOTHESIS** that means are equal.



ANOVA: Analysis of Variance is a variability ratio

$$\frac{\text{LARGE}}{\text{small}} = \text{Reject } H_0$$

At least one mean is an outlier and each distribution is narrow; distinct from each other

$$\frac{\text{Variance Between}}{\text{Variance Within}} \quad \frac{\text{similar}}{\text{similar}} = \text{Failed to reject } H_0$$

Means are fairly close to overall mean and/or distributions overlap a bit; hard to distinguish

$$\frac{\text{small}}{\text{LARGE}} = \text{αποτυχία απόρριψης της } H_0$$

The means are very close to the overall mean and/or distributions “melt” together

ANOVA: Analysis of Variance is a variability ratio

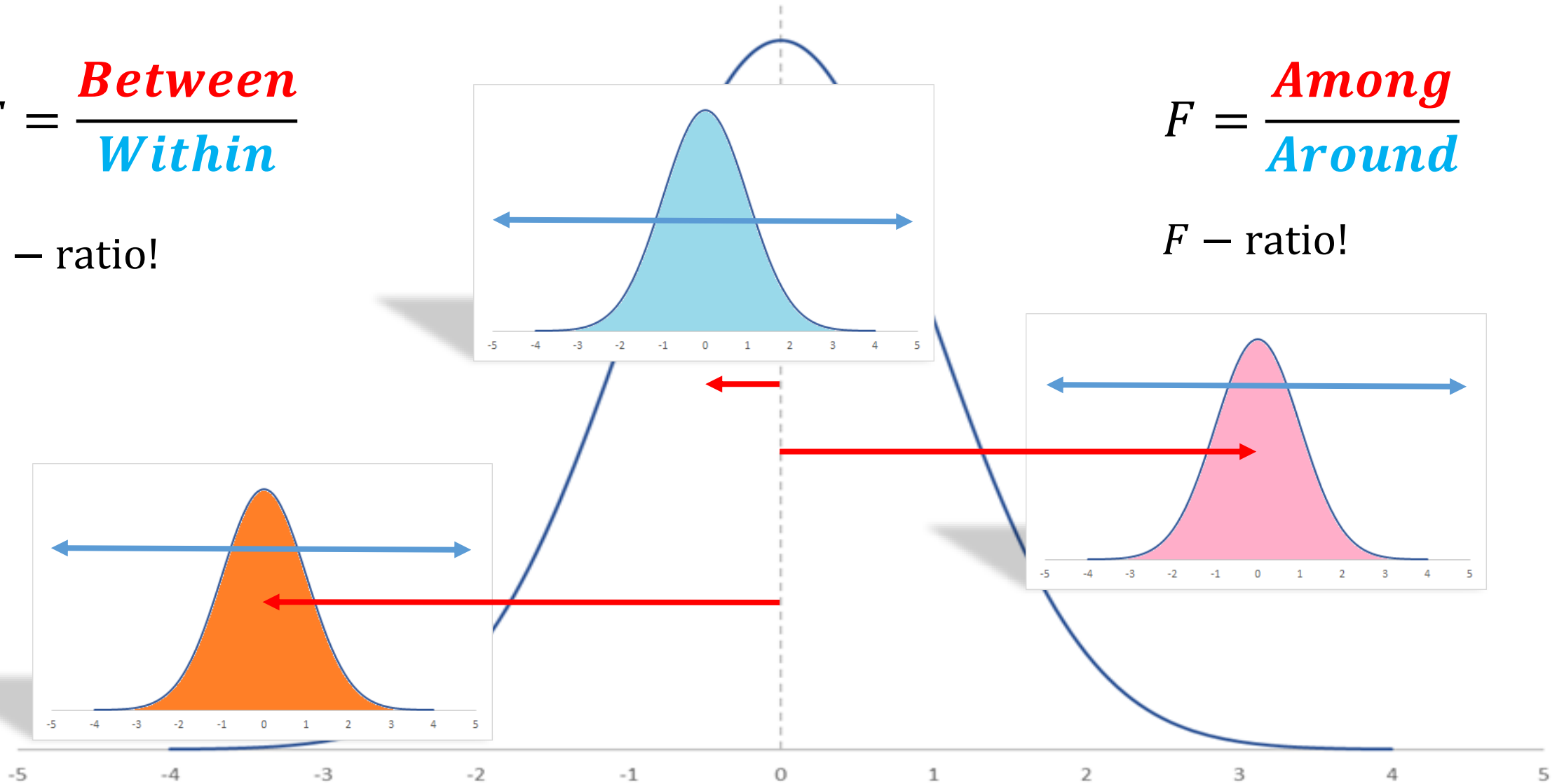
$$\text{Variance Between} + \text{Variance Within} = \text{Total Variance}$$

$$F = \frac{\text{Between}}{\text{Within}}$$

F – ratio!

$$F = \frac{\text{Among}}{\text{Around}}$$

F – ratio!

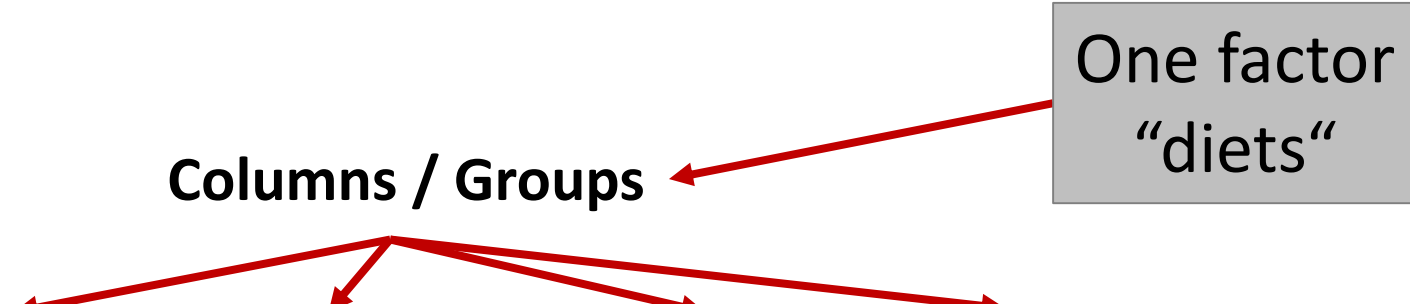




Why ANOVA;

- Up to this point, we have been comparing two populations
 - Independent samples t-test
 - Paired samples t-test
- Of course limiting ourselves to the comparison of two populations is well ... limiting
- What if we wish to compare the means of more than two populations?
- What if we wish to compare populations each containing several subgroups?
- For this reasons, we will make use of ANOVA (**A**nalysis **o**f **V**ariance)

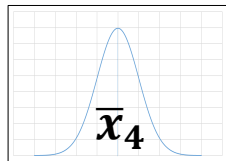
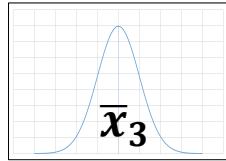
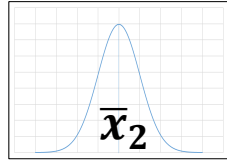
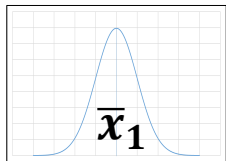
In a study, the liver weight (expressed as a percentage of body weight) of mice from four groups, each fed a different diet, was recorded. We aim to investigate whether there are systematic differences between the four groups



Columns / Groups

	a	b	c	d	
	3.42	3.17	3.34	3.64	
	3.96	3.63	3.72	3.93	
	3.87	3.38	3.81	3.77	
	4.19	3.47	3.66	4.18	
	3.58	3.39	3.55	4.21	
	3.76	3.41	3.51	3.88	
Mean	3.80	3.41	3.60	3.94	3.69 (total)

Sources of variability



	a	b	c	d
	3.42	3.17	3.34	3.64
	3.96	3.63	3.72	3.93
	3.87	3.38	3.81	3.77
	4.19	3.47	3.66	4.18
	3.58	3.39	3.55	4.21
	3.76	3.41	3.51	3.88
Mean	$\bar{x}_1 = 3.80$	$\bar{x}_2 = 3.41$	$\bar{x}_3 = 3.60$	$\bar{x}_4 = 3.94$

Overall mean:

**The average value of
all liver weights**

$$\bar{\bar{x}} = 3.69$$

- Testing for differences between groups relies on identifying all the sources that contribute to variability in the data (i.e., what makes the 24 numbers different)
- Therefore, the overall variability is broken down into individual sources of variation (this process is known as analysis of variance, or ANOVA).



Sources of variability

	a	b	c	d	
	3.42	3.17	3.34	3.64	
	3.96	3.63	3.72	3.93	
	3.87	3.38	3.81	3.77	
	4.19	3.47	3.66	4.18	
	3.58	3.39	3.55	4.21	
	3.76	3.41	3.51	3.88	
Mean	3.80	3.41	3.60	3.94	3.69 (total)

- Obviously, one source of variation is the effect of the 4 diets
- Another source is the within-group variation, as each mouse reacts differently to the same diet. This variability cannot be controlled and is therefore considered random error or random variation



Variance revisited and Sum of Squares

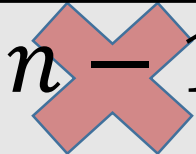
- Given that ANOVA is by definition the "analysis of variance," let's take a moment to go over variance in general
- The average squared deviation, or difference, between a data point and the distribution mean is called **variance**
 - Take the distance of each data point from the mean, square each distance, add them together, and then find the average
- If we take out the "find the average" part we are left with just the **SUM OF SQUARES (SS)**
- So SUM OF SQUARES is the variance without finding the average of the sum of squared deviations



Variance revisited and Sum of Squares

Sample Variance

$$s^2 = \frac{\sum (x - \mu)^2}{n - 1}$$

$$s^2 = \frac{\sum (x - \mu)^2}{n - 1}$$


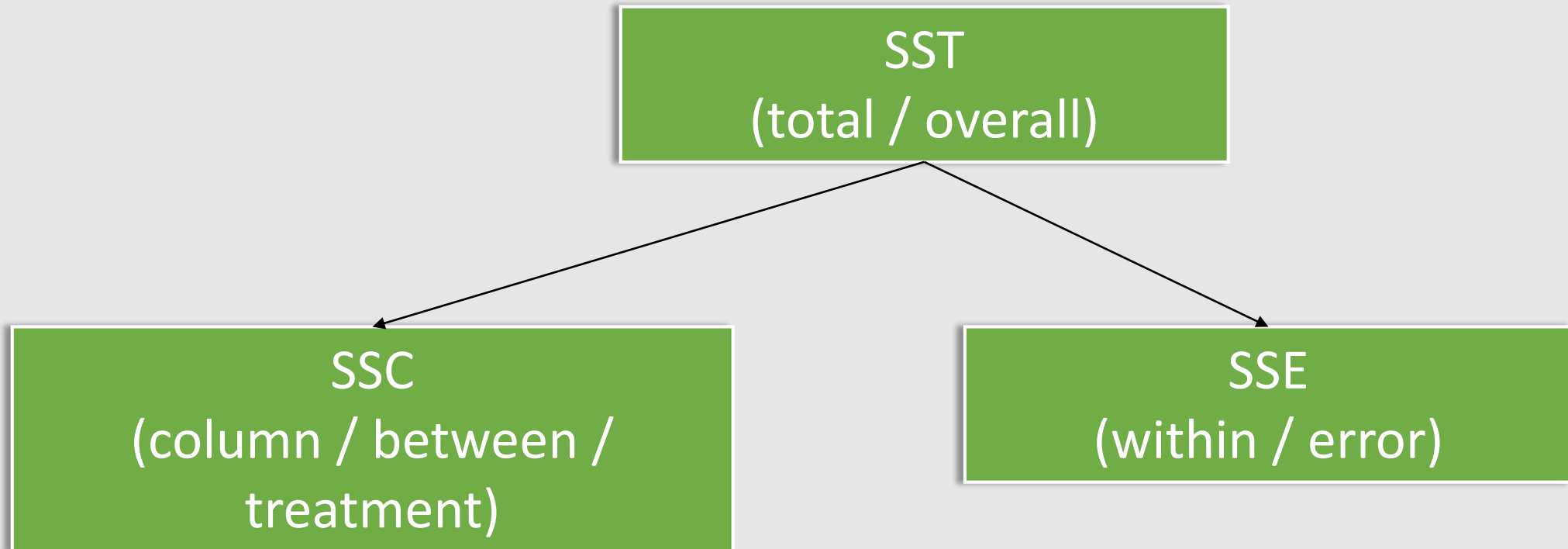
Sum of squares

$$SS = \sum (x - \mu)^2$$





Splitting Sum of Squares





Calculation of the SST sum of squares total

	A	B	C	D	E
1		a	b	c	d
2		3.42	3.17	3.34	3.64
3		3.96	3.63	3.72	3.93
4		3.87	3.38	3.81	3.77
5		4.19	3.47	3.66	4.18
6		3.58	3.39	3.55	4.21
7		3.76	3.41	3.51	3.88
8					
9	Mean	3.7966667	3.40833	3.59833	3.935

Overall mean:
The average value of all values is $\bar{x} = 3.69$

Calculation of the SST

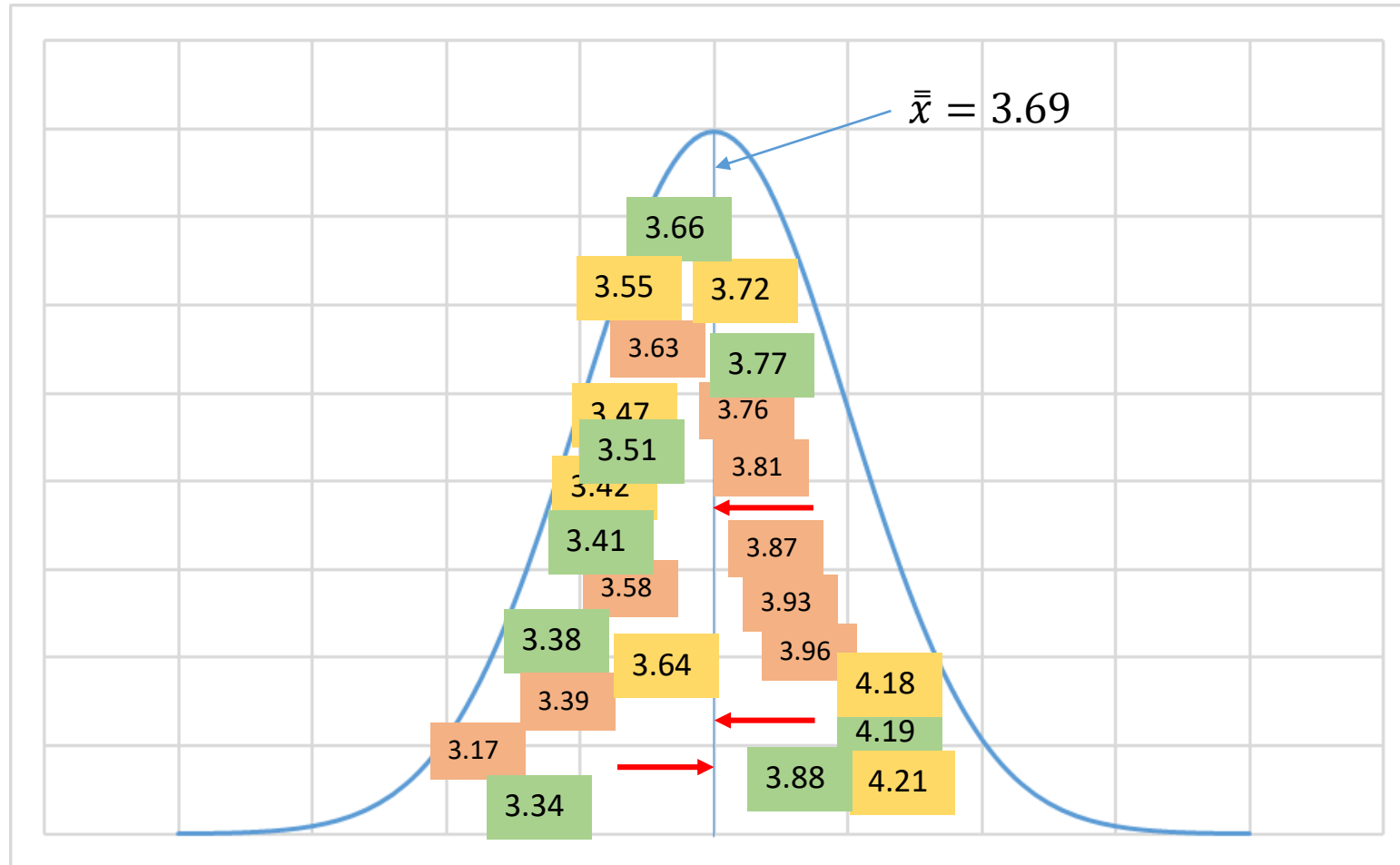
1. Find the difference between each data point and the overall mean
2. Square the difference
3. Add them up

`=AVERAGE(B2:B7)`

`=VAR.S(B2:E7)*23`

1.835196

SST (total / overall)



Calculation of the SST

1. Find the difference between each data point and the overall mean
2. Square the difference
3. Add them up

Squared distance is actually a SQUARE



Calculation of SSC (between groups) sum of square columns

	A	B	C	D	E
1		a	b	c	d
2		3.42	3.17	3.34	3.64
3		3.96	3.63	3.72	3.93
4		3.87	3.38	3.81	3.77
5		4.19	3.47	3.66	4.18
6		3.58	3.39	3.55	4.21
7		3.76	3.41	3.51	3.88
8					
9	Mean	3.7966667	3.40833	3.59833	3.935

Overall mean:

The average value of all values

$$\bar{x} = 3.69$$

Calculation of the SSC

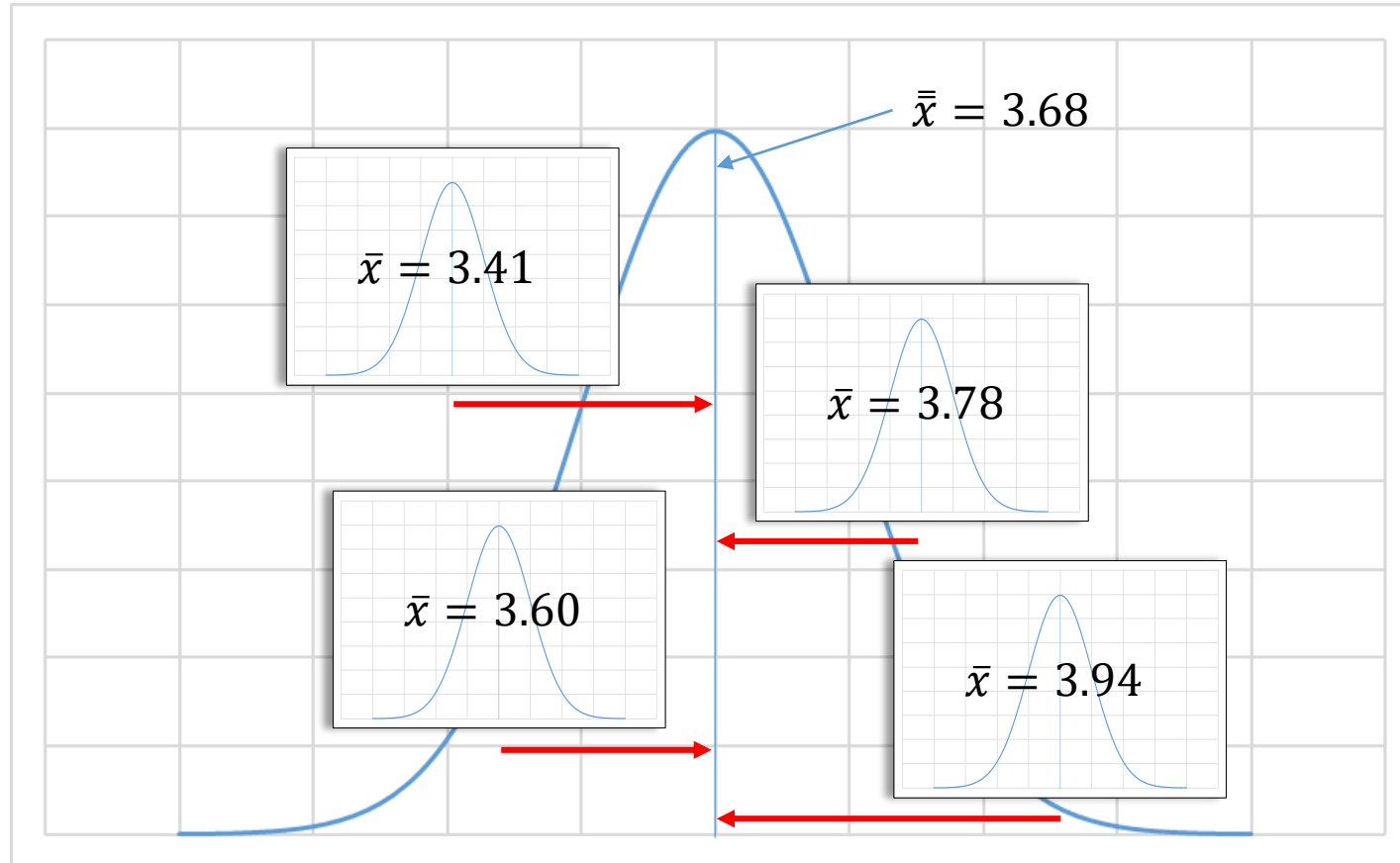
1. Find the difference between each group mean and the overall mean
2. Square the deviations
3. Multiply the squared differences by the number of observations
4. Add them up

`=AVERAGE(B2:B7)`

`=VAR.S(B9:E9)*3*6`

0.954145833

SSC
(column / between /
treatment)
sum of squares



Calculation of the
SSC

1. Find the difference between each group mean and the overall mean
2. Square the deviations
3. Multiply the squared differences by the number of observations
4. Add them up



Calculation of the SSE (within/error) sum of squares

	A	B	C	D	E
1		a	b	c	d
2		3.42	3.17	3.34	3.64
3		3.96	3.63	3.72	3.93
4		3.87	3.38	3.81	3.77
5		4.19	3.47	3.66	4.18
6		3.58	3.39	3.55	4.21
7		3.76	3.41	3.51	3.88
8					
9	Mean	3.7966667	3.40833	3.59833	3.935

~~$\bar{x} = 3.69$~~

Calculation of the SSE

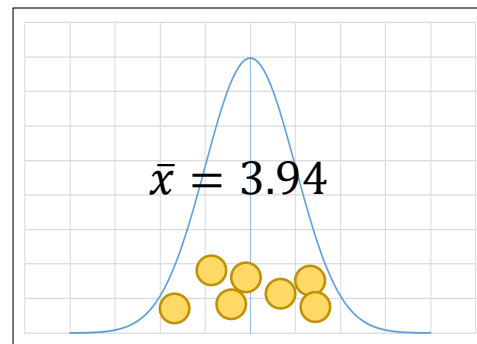
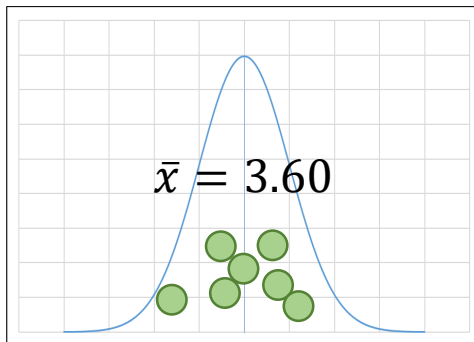
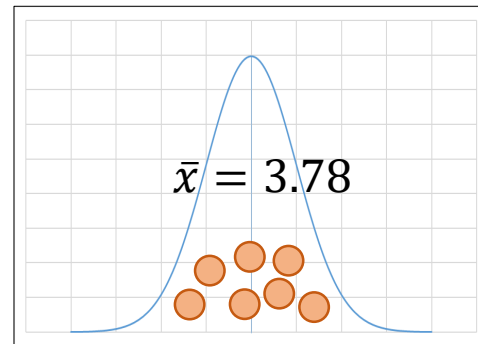
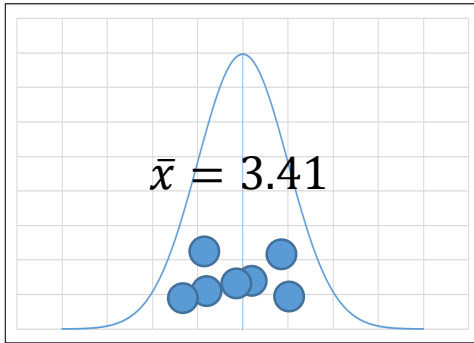
1. Find the difference between each data point and its own column mean
2. Square each deviation
3. Add them up
4. Add step 3 for all columns

```
=AVERAGE(B2:B7)
```

```
0.88105  
=VAR.S(B2:B7)*5+VAR.S(C2:C7)*5+VAR.S(D2:D7)*5+VAR.S(E2:E7)*5
```

SSE

(within / error)
sum of squares



Calculation of the SSE

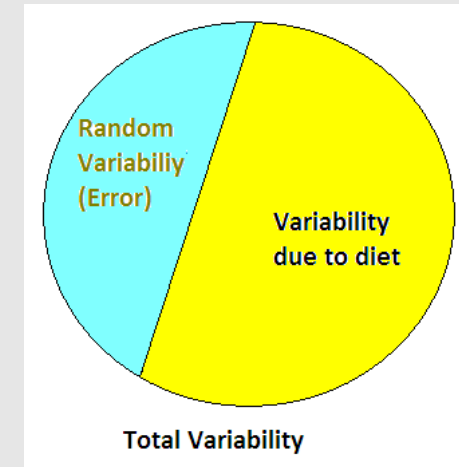
1. Find the difference between each data point and its own column mean
2. Square each deviation
3. Add them up
4. Add step 3 for all columns



One Way ANOVA

ANOVA is presented in the following table:

Source of variation (Πηγή μεταβλητότητας)	df (Βαθμοί ελευθερίας)	SS (Sum of squares)
Between groups (Μεταξύ των ομάδων)	4-1=3 (C-1)	0.954
Within groups (Εντός των ομάδων) (error/random)	24-4=20 (N-C)	0.881
Total	24-1=23 (N-1)	1.83



weight	ANOVA				
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.954	3	.318	7.220	.002
Within Groups	.881	20	.044		
Total	1.835	23			

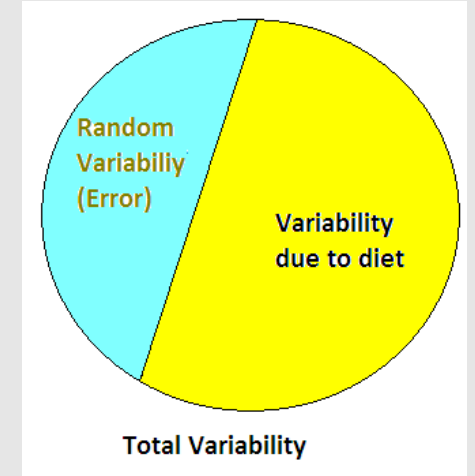
N = number of observations, **C** = #columns/groups/treatments/diets



One Way ANOVA

Then, the **mean square (MS)** for each source of variation is calculated using the formula $MS=SS/df$

Source of variation	df	SS	MS=SS/df (mean squares)
Between groups	3	0.954	0.318
Within groups (error/random)	20	0.881	0.044=s ²
Total	23	1.83	



weight	ANOVA				
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.954	3	.318	7.220	.002
Within Groups	.881	20	.044		
Total	1.835	23			



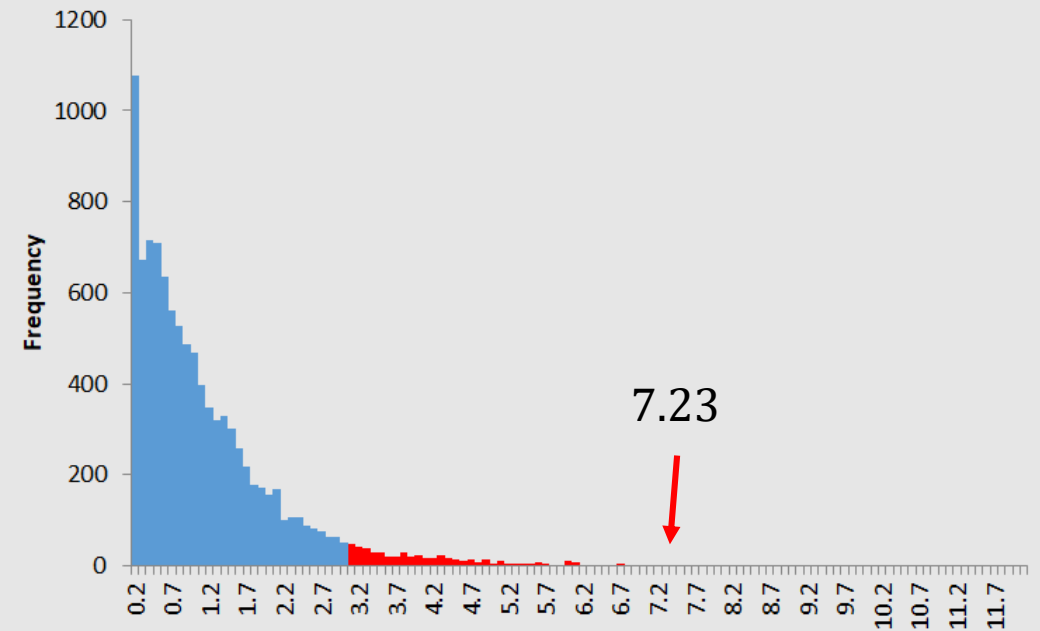
F – test

- Then, the between-group mean square is compared with the error-mean square, i.e. the within variance of each column, using the ***F – Test***:
- $$F = \frac{\text{Between group MS}}{\text{Error MS}} = \frac{0.318}{0.044} = 7.23$$
- If the **variability BETWEEN** the four group means in the numerator is larger compared to the variance **WITHIN** the four groups (internal spread) in the denominator, the differences between the four groups are not random; they are real
- In this case, the ***F – Test*** value, is way larger than 1



Significance

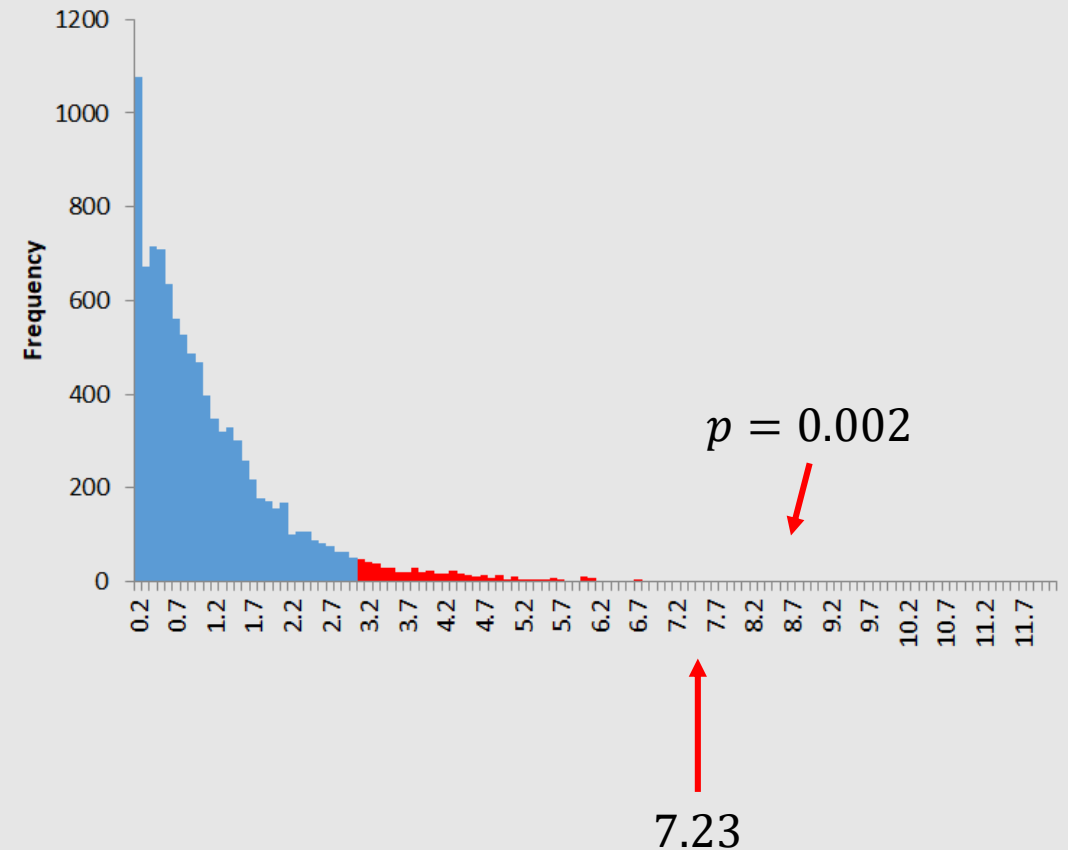
- The significance of the value $F = 7.23$ is determined in a similar way to a t – $test$
- We randomly simulate the study 10.000 times and calculate the F – $tests$ each time. The 10.000 F – $tests$ then form the F – distribution
- Finally, we find the percentage of F – $tests$ that are greater than $F = 7.23$





Significance

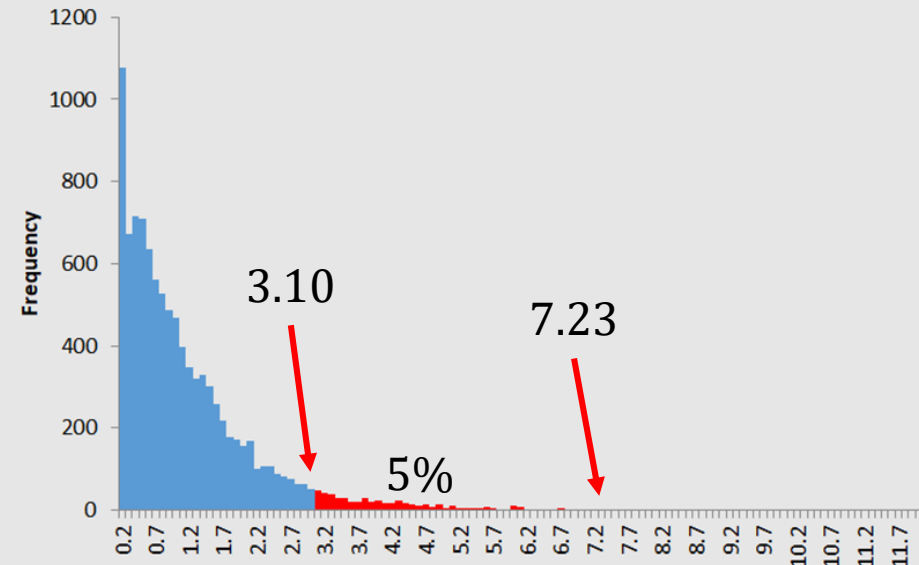
- The percentage of F-tests which are greater than $F = 7.23$ is $P = 0.002$
- Thus, the four diets are statistically different with a small probability error ($P < 0.05$ $\hat{=}$ $P = 0.002$)





F – test

- Alternatively, the value $F = 7.23$ is compared to the 5% point of the F-distribution with 3 and 20 degrees of freedom which is 3.1 (see F-distribution table in the next slide)
- Because $F = 7.23$ greater than 3.1, we conclude that there is an indication ($P < 0.05$) that the diets differ from each other statistically significant



Εύρεση του 5% σημείου της F κατανομής για 3 και 20 df στο Excel

```
=F.INV.RT(0.05, 3, 20)
```

```
3.098391
```

Table of f – distribution

Degrees of freedom in denominator	Degrees of freedom in numerator												
	1	2	3	4	5	6	7	8	9	10	20	40	∞
1	161.40	199.50	215.70	224.60	230.20	234.00	236.80	238.90	240.50	241.90	248.00	251.10	254.30
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.45	19.47	19.50
3	10.13	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81	8.79	8.66	8.59	8.50
4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.80	5.72	5.60
5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.56	4.46	4.40
6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	3.87	3.77	3.70
7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.44	3.34	3.20
8	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.15	3.04	2.90
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	2.94	2.83	2.70
10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.77	2.66	2.50
11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.85	2.65	2.53	2.40
12	4.75	3.89	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75	2.54	2.43	2.30
13	4.67	3.81	3.41	3.18	3.03	2.92	2.83	2.77	2.71	2.67	2.46	2.34	2.20
14	4.60	3.74	3.34	3.11	2.96	2.85	2.76	2.70	2.65	2.60	2.39	2.27	2.10
15	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.33	2.20	2.10
16	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49	2.28	2.15	2.00
17	4.45	3.59	3.20	2.96	2.81	2.70	2.61	2.55	2.49	2.45	2.23	2.10	2.00
18	4.41	3.55	3.16	2.93	2.77	2.66	2.58	2.51	2.46	2.41	2.19	2.06	1.90
19	4.38	3.52	3.13	2.90	2.74	2.63	2.54	2.48	2.42	2.38	2.16	2.03	1.90
20	4.35	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35	2.12	1.99	1.80
30	4.17	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21	2.16	1.93	1.79	1.60
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	2.12	2.08	1.84	1.69	1.50
120	3.92	3.07	2.68	2.45	2.29	2.18	2.09	2.02	1.96	1.91	1.66	1.50	1.30
∞	3.84	3.00	2.60	2.37	2.21	2.10	2.01	1.94	1.88	1.83	1.57	1.39	1.00

One Way ANOVA

Post Hoc Test



Multiple comparison procedures

- The ANOVA test only tells us if all the population means are equal (are likely to come from the same population)
- If the F-test is significant, we do not know where the differences are located however
- It is necessary to compare each population pair::
 - A, B, C the pairings would be AB, AC, and BC or $C(3, 2) = \frac{3!}{(3-2)!2!} = \frac{1 \times 2 \times 3}{1 \times (1 \times 2)} = \frac{6}{2} = 3$ pairs
 - A, B, C, D the pairings would be AB, AC, AD, BC, BD, and CD or $C(4, 2) = 6$ pairs
- There are several multiple comparison tests: Fisher's, LSD, Tukey HSD, Bonferroni



Multiple comparison procedures

	Groups / Diets			
	a	b	c	d
Mean	3.80	3.41	3.60	3.94
Standard deviation	0.27	0.15	0.17	0.22
Count	6	6	6	6

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.954145833	3	0.318048611	7.219763035	0.001805552	3.098391212
Within Groups	0.88105	20	0.0440525			
Total	1.835195833	23				



Difference Matrix

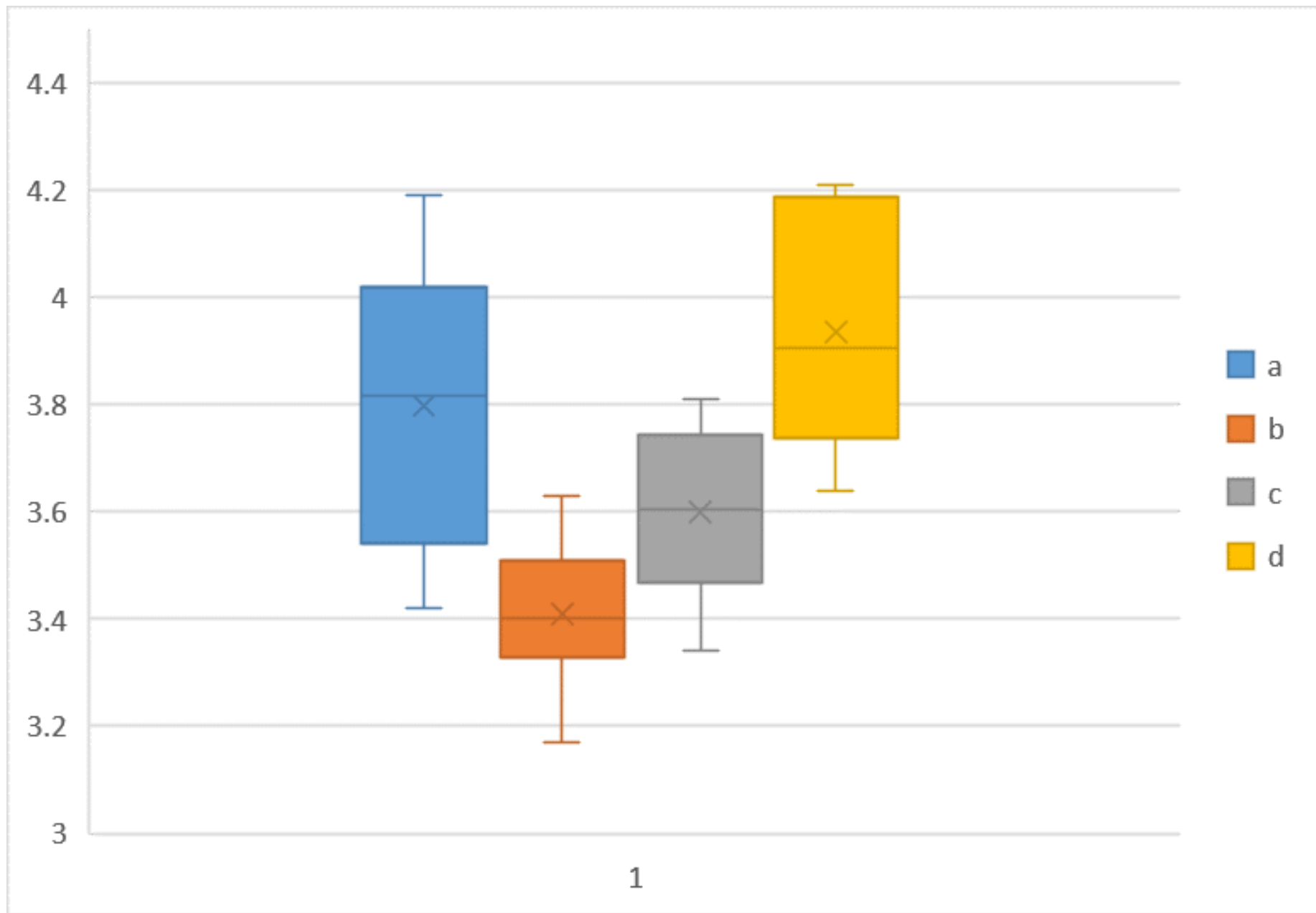
	d	a	c	b
d	0	0.14	0.34	0.53
a	-0.14	0	0.2	0.39
c	-0.34	-0.2	0	0.19
b	-0.53	-0.39	-0.19	0



Six pairwise comparisons

	Mean #1	Mean #2	Difference
a vs b	3.80	3.41	0.39
a vs c	3.80	3.60	0.20
a vs d	3.80	3.94	-0.14
b vs c	3.41	3.60	-0.19
b vs d	3.41	3.94	-0.53
c vs d	3.60	3.94	-0.34

Which of these pairwise comparisons contain statistically significant differences?





Bonferroni procedure

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.954145833	3	0.318048611	7.219763035	0.001805552	3.098391212
Within Groups	0.88105	20	0.0440525			
Total	1.835195833	23				

$$H_0: \mu_i = \mu_j$$
$$H_\alpha: \mu_i \neq \mu_j$$
$$t = \frac{\bar{x}_i - \bar{x}_j}{\sqrt{MSE \left(\frac{1}{n_i} + \frac{1}{n_j} \right)}}$$
$$SE = \sqrt{MSE \left(\frac{1}{n_i} + \frac{1}{n_j} \right)}$$



a vs b

$$t = \frac{3.80 - 3.41}{\sqrt{0.044 \left(\frac{1}{6} + \frac{1}{6} \right)}}$$

$$t = \frac{0.39}{0.1225}$$

$$t = 3.1837$$

$$t = \frac{0.39}{\sqrt{0.044(0.33)}}$$

20 degrees of freedom

$$t = \frac{0.39}{\sqrt{0.015}}$$

$$= \mathbf{T.DIST.2T(3.1837, 20) = 0.004667}$$



a vs b

$$t = 3.1837 \quad =\text{T.DIST.2T}(3.1837, 20)=0.004667$$

- The value of the *t* – test ($t = 3.1837$) is greater than the 5% point of the *t* – distribution for 20 *df* (the *df* of the error) which is 2.09
- **Thus, there is a statistically significant difference between a and b ($p < 0.05$ or more precisely $p = 0.004$)**



95% confidence interval of the difference

$$\left((\bar{x}_a - \bar{x}_b) - t \times SE, D + t \times SE \right)$$

$$\left((3.80 - 3.41) - 2.09 \times 0.12, (3.80 - 3.41) + 2.09 \times 0.12 \right)$$

$$(0.39 - 0.251, 0.39 + 0.251)$$

$$(0.139, 0.641)$$

Thus, with 95% confidence, group a has between 0.14 and 0.64 more weight than group b. Since 0 is not included in the 95% confidence interval, the difference is statistically significant

	t distribution		
	Percentage points of the t distribution		
	p-value		
degrees of freedom	0.05	0.01	0.001
	two tails	two tails	two tails
1	12.71	63.66	636.62
2	4.30	9.92	31.60
3	3.18	5.84	12.92
4	2.78	4.60	8.61
5	2.57	4.03	6.87
6	2.45	3.71	5.96
7	2.36	3.50	5.41
8	2.31	3.36	5.04
9	2.26	3.25	4.78
10	2.23	3.17	4.59
20	2.09	2.85	3.85



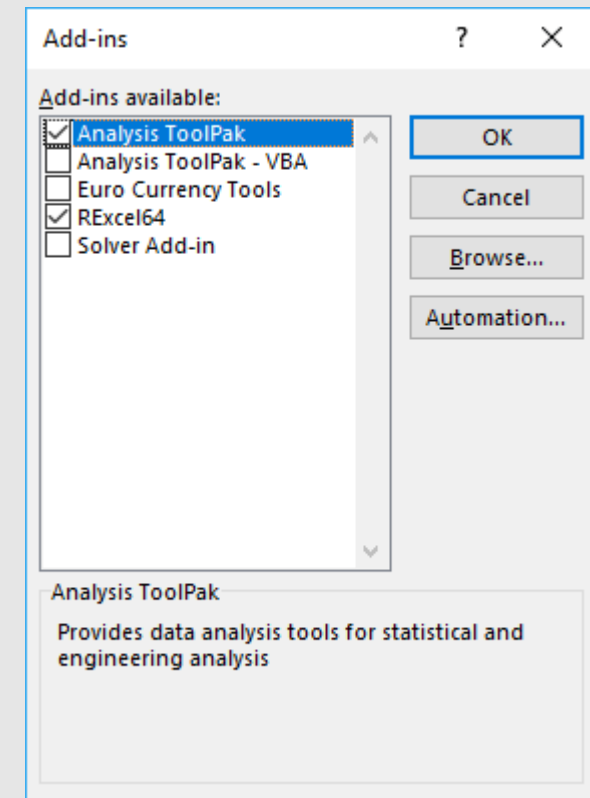
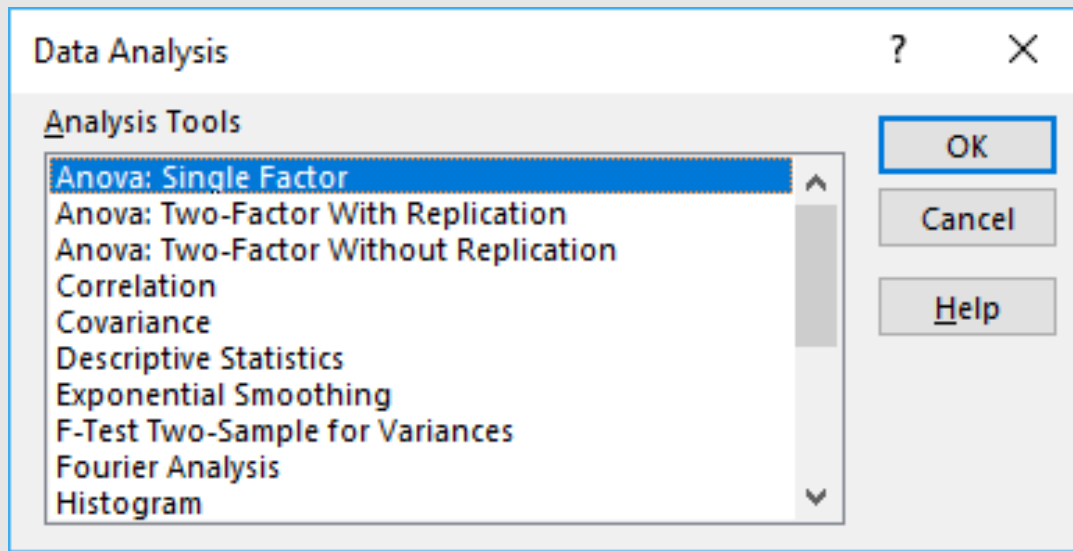
Bonferroni correction

- All possible comparisons between groups are not independent, and there is always the possibility of finding a false significant effect
- For this reason, when multiple comparisons (k) are made between groups the level of significance (P) must be corrected to $P' = k * P$
- Therefore, if we perform 6 comparisons between groups, the comparison between diet a and b will be significant at $P = 6 * 0.004667 = 0.028$



Excel Data Analysis

File -> Options -> Add Ins -> Go ... -> Analysis ToolPak





Excel Data Analysis

Data -> Data Analysis

	A	B	C	D	E	F	G	H	I
1	a	b	c	d					
2	3.42	3.17	3.34	3.64					
3	3.96	3.63	3.72	3.93					
4	3.87	3.38	3.81	3.77					
5	4.19	3.47	3.66	4.18					
6	3.58	3.39	3.55	4.21					
7	3.76	3.41	3.51	3.88					
8									
9									
10									
11									
12									

Data Analysis

Analysis Tools

- Anova: Single Factor
- Anova: Two-Factor With Replication
- Anova: Two-Factor Without Replication
- Correlation
- Covariance
- Descriptive Statistics
- Exponential Smoothing
- F-Test Two-Sample for Variances
- Fourier Analysis
- Histogram

OK Cancel Help



Excel Data Analysis

	A	B	C	D
1	a	b	c	d
2	3.42	3.17	3.34	3.64
3	3.96	3.63	3.72	3.93
4	3.87	3.38	3.81	3.77
5	4.19	3.47	3.66	4.18
6	3.58	3.39	3.55	4.21
7	3.76	3.41	3.51	3.88
8				
9				
10				
11				
12				
13				
14				

Anova: Single Factor

Input
Input Range:

Grouped By: Columns Rows

Labels in first row
Alpha:

Output options
 Output Range:
 New Worksheet Ply:
 New Workbook

OK Cancel Help



Excel Data Analysis

9	Anova: Single Factor						
10							
11	SUMMARY						
12	<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
13	a	6	22.78	3.79666667	0.07538667		
14	b	6	20.45	3.40833333	0.02217667		
15	c	6	21.59	3.59833333	0.02805667		
16	d	6	23.61	3.935	0.05059		
17							
18							
19	ANOVA						
20	<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
21	Between Groups	0.95414583	3	0.31804861	7.21976304	0.00180555	3.09839121
22	Within Groups	0.88105	20	0.0440525			
23							
24	Total	1.83519583	23				