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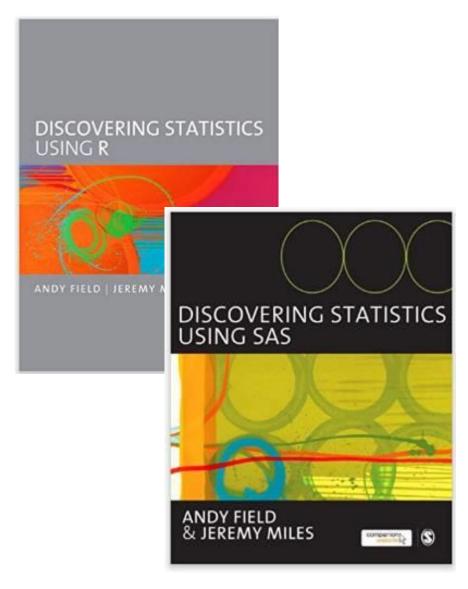
# One-way anova





#### References

- Discovering statistics using R" by Andy Field <u>http://www.uk.sagepub.com/dsur/main.htm</u>
- SAS course notes









#### **Aims**

- Understand the basic principles of ANOVA
  - Why it is done?
  - What it tells us?
- Theory of one-way independent ANOVA
- Following up an ANOVA:
  - Post hoc tests





## When and Why

- When we want to compare means we can use a T-test. This test has limitations:
  - You can compare only 2 means: often we would like to compare means from 3 or more groups.
  - It can be used only with one predictor/independent variable.

#### ANOVA

- Compares several means.
- Can be used when you have manipulated more than one independent variable.
- It is an extension of regression (the general linear model).

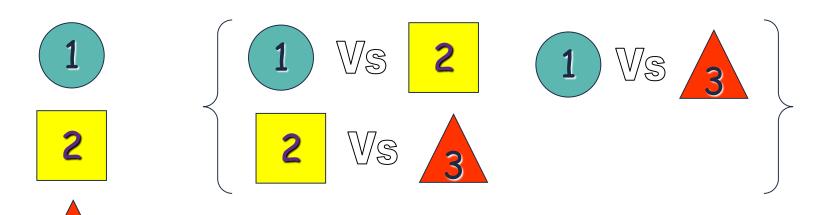




## Why Not Use Lots of *T*-Tests?



- If we want to compare several means why don't we compare pairs of means with *T*-tests?
  - Can't look at several independent variables.
  - Inflates the Type I error rate.



familywise error =  $1 - 0.95^n$ 





# **Multiple Comparisons**

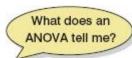
| Comparisonwise<br>Error Rate<br>(α=0.05) | Number of Comparisons | Experimentwise<br>Error Rate<br>(α=0.05) |
|--|-----------------------|--|
| .05                                      | 1                     | .05                                      |
| .05                                      | 3                     | .14                                      |
| .05                                      | 6                     | .26                                      |
| .05                                      | 10                    | .40                                      |





## What Does ANOVA Tell Us?

- Null hyothesis:
  - Like a T-test, ANOVA tests the null hypothesis that the means are the same.
- Experimental hypothesis:
  - The means differ.
- ANOVA is an omnibus test
  - It test for an overall difference between groups.
  - It tells us that the group means are different.
  - It doesn't tell us exactly which means differ.









## **Assumptions ANOVA**

- 1. The measure is interval-level continuous data.
- 2. The measure is normally distributed (within each group), *i.e.*, Y|X is normally distributed.
- 3. The variance of the measure is the same in each group.
- 4. The observations are independent

Note that 2,3 and 4 are the same as saying

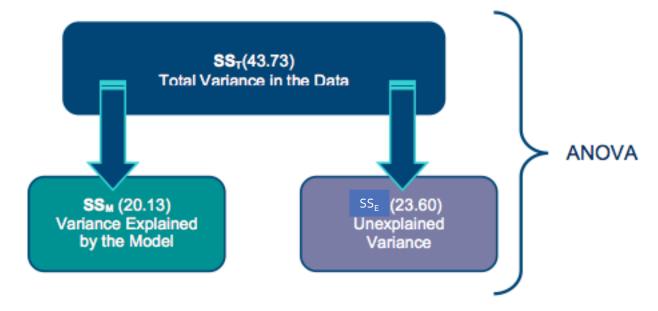
$$\epsilon_i \sim i.i.d.N(0,\sigma^2)$$





## Theory of ANOVA

- If the experiment is successful, then the model will explain more variance than it can't
  - SS<sub>M</sub> will be greater than SS<sub>E</sub>







## **ANOVA** by Hand

- Testing the effects of Viagra on libido using three groups:
  - Placebo (sugar pill)
  - Low dose viagra
  - High dose viagra
- The outcome/dependent variable (DV) was an objective measure of libido.







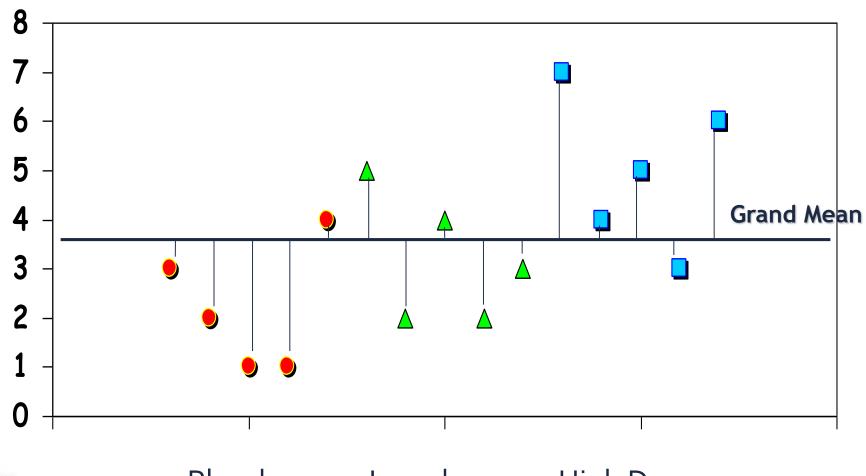
## The Data

Table 10.1: Data in Viagra.dat

|   | Placebo  | Low Dose | High Dose |  |  |  |  |  |  |
|---|--|----------|-----------|--|--|--|--|--|--|
| $x_{ij}$  | 3  | 5        | 7         |  |  |  |  |  |  |
|   | 2  | 2        | 4         |  |  |  |  |  |  |
|   | 1  | 4        | 5         |  |  |  |  |  |  |
|   | 1  | 2        | 3         |  |  |  |  |  |  |
|   | 4  | 3        | 6         |  |  |  |  |  |  |
| $x_{.j}^{-}$  | 2.20   | 3.20     | 5.00      |  |  |  |  |  |  |
| $s_{j}$   | 1.30   | 1.30     | 1.58      |  |  |  |  |  |  |
| $egin{array}{c} x_{.j}^- \ s_j \ s_j^2 \ \end{array}$ | 1.70   | 1.70     | 2.50      |  |  |  |  |  |  |
| Gi  | Grand Mean = 3.467 Grand SD = 1.767 $\mathcal{X}$ Grand Variance = 3.124 |          |           |  |  |  |  |  |  |



## Total Sum of Squares (SS<sub>T</sub>):





## Step 1: Calculate SS<sub>T</sub>

$$SS_{T} = \sum_{i=1}^{N} (x_{i} - \bar{x}_{..})^{2}$$

$$s^{2} = \frac{SS}{N-1}$$

$$SS = s^{2}(N-1)$$

$$SS_{T} = s^{2}(N-1)$$

$$SS_{T} = 3.124(15-1)$$

$$SS_{T} = 43.74$$





## **Degrees of Freedom**

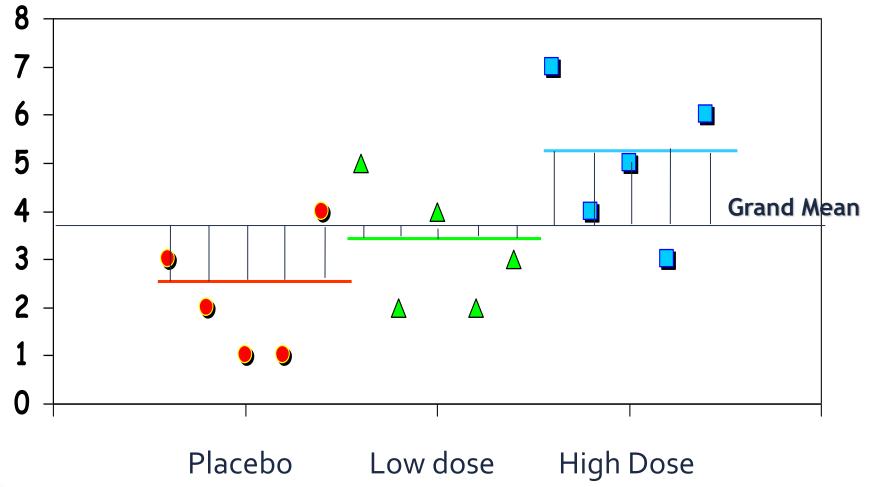
- Degrees of freedom (*df*) are the number of values that are free to vary.
- In general, the *df* are one less than the number of values used to calculate the SS.

$$df_T = N - 1 = 15 - 1 = 14$$





# Model Sum of Squares (SS<sub>M</sub>):





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# Step 2: Calculate SS<sub>M</sub>

$$SS_M = \sum_{j=1}^k n_j (\bar{x}_{.j} - \bar{x}_{..})^2$$



$$SS_M = 5(2.2 - 3.467)^2 + 5(3.2 - 3.467)^2 + 5(5.0 - 3.467)^2$$
  
 $SS_M = 20.135$ 





## **Model Degrees of Freedom**

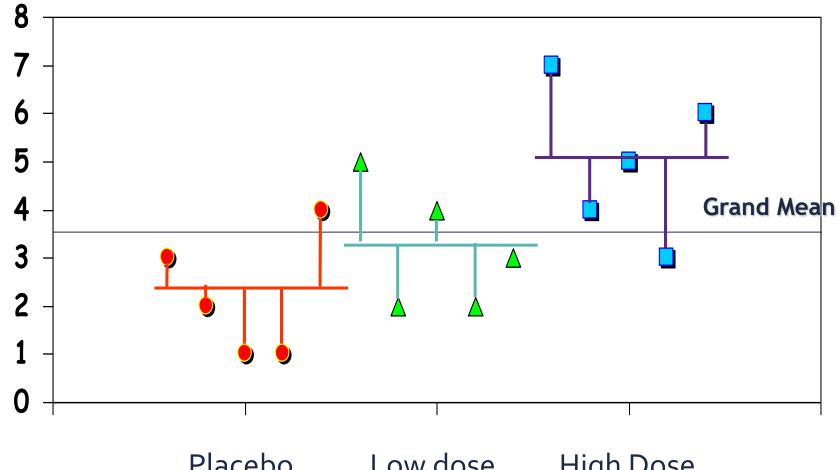
- How many values did we use to calculate SS<sub>M</sub>?
  - We used the 3 means.

$$df_M = k - 1 = 3 - 1 = 2$$





## Residual Sum of Squares (SS<sub>E</sub>):







Placebo

Df = 4

Low dose

Df = 4

High Dose

Df = 4

# Step 3: Calculate SS<sub>E</sub>

$$SS_E = \sum_{j=1}^{k} \sum_{i=1}^{N} (x_{ij} - \bar{x}_{.j})^2$$

$$s^2 = \frac{SS}{N-1}$$

$$SS = s^2(N-1)$$

$$SS_E = \sum_{j=1}^k s_j^2(n_j - 1)$$
  

$$SS_E = s_1^2(n_1 - 1) + s_2^2(n_2 - 1) + s_3^2(n_3 - 1)$$





## Step 3: Calculate SS<sub>E</sub>

$$SS_E = s_1^2(n_1 - 1) + s_2^2(n_1 - 1) + s_3^2(n_1 - 1)$$

$$= 1.70(5 - 1) + 1.70(5 - 1) + 2.50(5 - 1)$$

$$= 23.60$$





## Residual Degrees of Freedom

- How many values did we use to calculate SS<sub>E</sub>?
  - ▶ We used the 5 scores for each of the SS for each group.

$$df_E = df_1 + df_2 + df_3$$
  

$$df_E = (n_1 - 1) + (n_2 - 1) + (n_3 - 1)$$
  

$$df_E = N - k = 12$$





## **Double Check**

$$SS_T = SS_M + SS_E$$
  
 $43.74 = 20.14 + 23.60$   
 $43.74 = 43.74$ 

$$df_T = df_M + df_E$$
$$14 = 2 + 12$$





## Step 4: Calculate the Mean Squares

$$MS_M = \frac{SS_M}{df_M} = \frac{20.135}{2} = 10.067$$
  
 $MS_E = \frac{SS_E}{df_E} = \frac{23.60}{12} = 1.967$ 





## Step 5: Calculate the F-Ratio

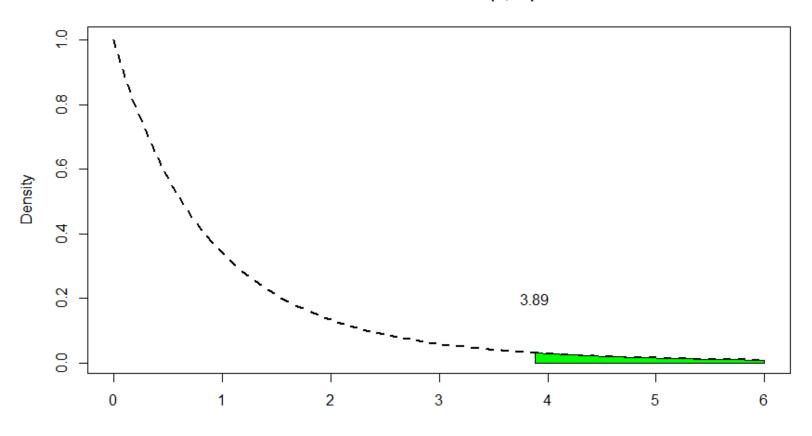
$$F = \frac{MS_M}{MS_E} = \frac{10.067}{1.967} = 5.12$$





## F Statistic and Critical Values at $\alpha$ =0.05

#### F-distribution df=(2,12)











## Step 6: Construct a Summary Table

| Source   | SS    | df | MS     | F     |
|----------|-------|----|--------|-------|
| Model    | 20.14 | 2  | 10.067 | 5.12* |
| Residual | 23.60 | 12 | 1.967  |       |
| Total    | 43.74 | 14 |        |       |





## Workflow

- Summary statistics
- Graphs (boxplots, barcharts)
- Statistical model
- Test the assumptions
- Post-hoc tests





## One-Way ANOVA using SAS: output

#### analysis of variance with Levene's test for equality of variances

#### The GLM Procedure

#### Dependent Variable: libido

| Source          | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|-----------------|----|----------------|-------------|---------|--------|
| Model           | 2  | 20.13333333    | 10.06666667 | 5.12    | 0.0247 |
| Error           | 12 | 23.60000000    | 1.96666667  |         |        |
| Corrected Total | 14 | 43.73333333    |             |         |        |

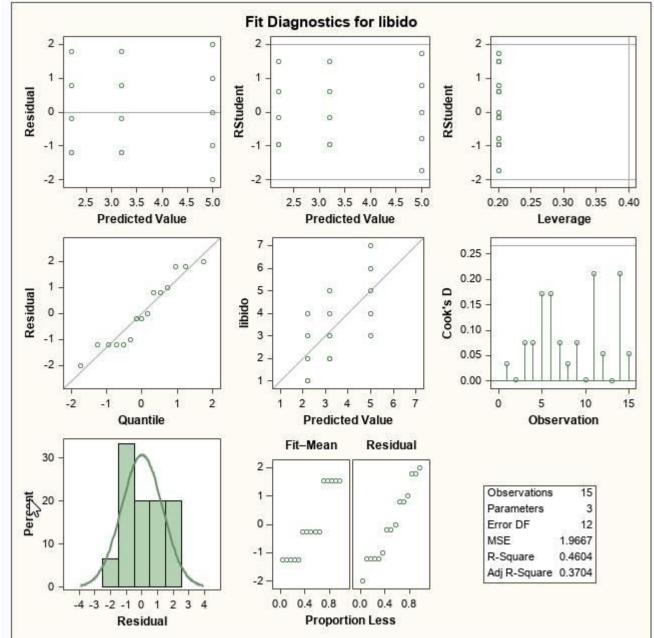
| R-Square | Coeff Var | Root MSE | libido Mean |  |  |
|----------|-----------|----------|-------------|--|--|
| 0.460366 | 40.45324  | 1.402379 | 3.466667    |  |  |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |  |
|--------|----|-------------|-------------|---------|--------|--|
| dose   | 2  | 20.13333333 | 10.06666667 | 5.12    | 0.0247 |  |





# Verifying the assumptions









## Why Use Follow-Up Tests?

- The F-ratio tells us only that the experiment was successful
  - i.e. group means were different
- It does not tell us specifically which group means differ from which.
- We need additional tests to find out where the group differences lie.

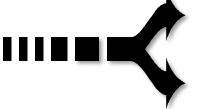




## **Multiple Comparison Methods**

Control
Comparisonwise
Error Rate
Pairwise t-tests

Control Experimentwise Error Rate



Compare All Pairs
Tukey

Compare to Control
Dunnett





#### **Post Hoc Tests**

- Compare each mean against all others.
- In general terms they use a stricter criterion to accept an effect as significant.
  - Hence, control the familywise error rate.
  - Simplest example is the Bonferroni method:

$$Bonferroni \alpha = \frac{\alpha}{\text{number of tests}}$$





## **Tukey's Multiple Comparison Method**

- This method is appropriate when you consider pairwise comparisons only.
- The experimentwise error rate is
  - equal to alpha when all pairwise comparisons are considered
  - less than alpha when *fewer* than all pairwise comparisons are considered.







## Special Case of Comparing to a Control

- Comparing to a control is appropriate when there is a natural reference group, such as a placebo group in a drug trial.
  - ▶ Control comparison computes and tests *k*-1 groupwise differences, where *k* is the number of levels of the classification variable.
  - An example is the *Dunnett* method.





## Output all-pairwise comparisons

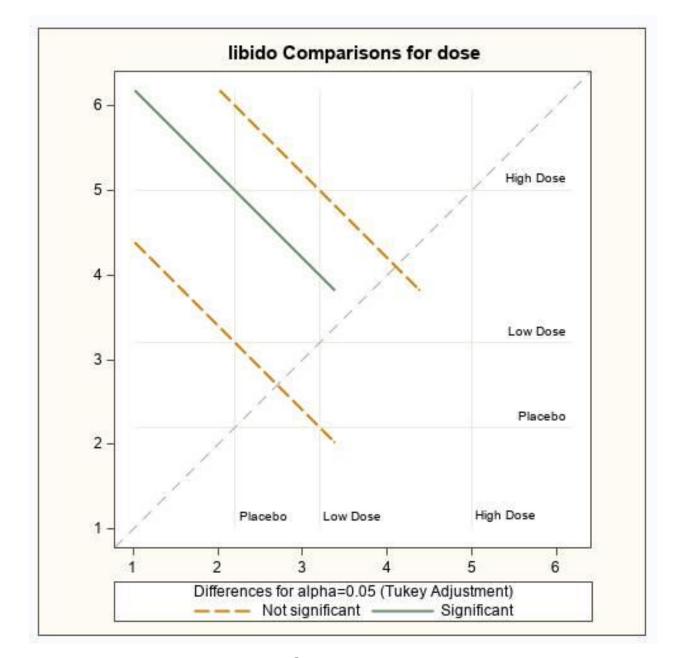
| dose Least Squares Means |          |                |    |         |         |       |        |        |  |
|--------------------------|----------|----------------|----|---------|---------|-------|--------|--------|--|
| dose                     | Estimate | Standard Error | DF | t Value | Pr >  t | Alpha | Lower  | Upper  |  |
| High Dose                | 5.0000   | 0.6272         | 12 | 7.97    | <.0001  | 0.05  | 3.6335 | 6.3665 |  |
| Low Dose                 | 3.2000   | 0.6272         | 12 | 5.10    | 0.0003  | 0.05  | 1.8335 | 4.5665 |  |
| Placebo                  | 2.2000   | 0.6272         | 12 | 3.51    | 0.0043  | 0.05  | 0.8335 | 3.5665 |  |

| Differences of dose Least Squares Means Adjustment for Multiple Comparisons: Tukey |          |          |                |    |         |         |        |       |         |        |           |           |
|--|----------|----------|----------------|----|---------|---------|--------|-------|---------|--------|-----------|-----------|
| dose   | _dose    | Estimate | Standard Error | DF | t Value | Pr >  t | Adj P  | Alpha | Lower   | Upper  | Adj Lower | Adj Upper |
| High Dose  | Low Dose | 1.8000   | 0.8869         | 12 | 2.03    | 0.0652  | 0.1475 | 0.05  | -0.1325 | 3.7325 | -0.5662   | 4.1662    |
| High Dose  | Placebo  | 2.8000   | 0.8869         | 12 | 3.16    | 0.0083  | 0.0209 | 0.05  | 0.8675  | 4.7325 | 0.4338    | 5.1662    |
| Low Dose   | Placebo  | 1.0000   | 0.8869         | 12 | 1.13    | 0.2816  | 0.5163 | 0.05  | -0.9325 | 2.9325 | -1.3662   | 3.3662    |





# diffogram







#### Output comparisons vs a control

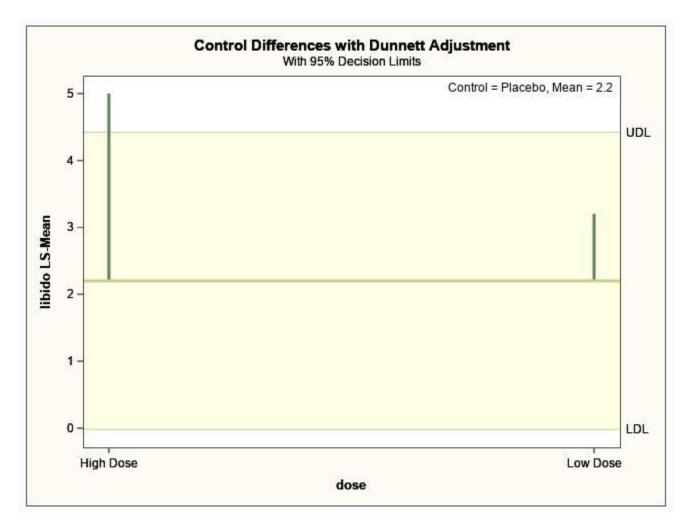
| dose Least Squares Means |          |                |    |         |         |       |        |        |  |  |
|--------------------------|----------|----------------|----|---------|---------|-------|--------|--------|--|--|
| dose                     | Estimate | Standard Error | DF | t Value | Pr >  t | Alpha | Lower  | Upper  |  |  |
| High Dose                | 5.0000   | 0.6272         | 12 | 7.97    | <.0001  | 0.05  | 3.6335 | 6.3665 |  |  |
| Low Dose                 | 3.2000   | 0.6272         | 12 | 5.10    | 0.0003  | 0.05  | 1.8335 | 4.5665 |  |  |
| Placebo                  | 2.2000   | 0.6272         | 12 | 3.51    | 0.0043  | 0.05  | 0.8335 | 3.5665 |  |  |

| Differences of dose Least Squares Means Adjustment for Multiple Comparisons: Dunnett |         |          |                |    |         |         |        |       |         |        |           |           |
|--|---------|----------|----------------|----|---------|---------|--------|-------|---------|--------|-----------|-----------|
| dose   | _dose   | Estimate | Standard Error | DF | t Value | Pr >  t | Adj P  | Alpha | Lower   | Upper  | Adj Lower | Adj Upper |
| High Dose  | Placebo | 2.8000   | 0.8869         | 12 | 3.16    | 0.0083  | 0.0152 | 0.05  | 0.8675  | 4.7325 | 0.5805    | 5.0195    |
| Low Dose   | Placebo | 1.0000   | 0.8869         | 12 | 1.13    | 0.2816  | 0.4459 | 0.05  | -0.9325 | 2.9325 | -1.2195   | 3.2195    |





# Output comparisons vs a control







# When variances are not equal across groups

- If Levene's test is significant then it is reasonable to assume that population variances are different across groups.
- Welch anova (variance weighted)
- Mixed models allow estimation of heterogeneous variances
- Unless the group variances are extremely different or the number of groups is large, the usual ANOVA test is relatively robust when the groups are all about the same size.





## Quiz: anova as regression

• In the viagra dataset we compare 3 groups. How many dummy variables do we have in the regression model?

- a) 1
- b) 2
- c) 3
- d) 4





#### Quiz: anova as regression

• In the viagra dataset we compare 3 groups. How many dummy variables doe we have in the regression model

- a) **1**
- b) 2
- c) 3
- d) 4





# **GLM Coding of CLASS Variables (default)**

| CLASS | Value     | X <sub>1</sub> | X <sub>2</sub> | $X_3$ |
|-------|-----------|----------------|----------------|-------|
| dose  | High Dose | 1              | 0              | 0     |
|       | Low Dose  | 0              | 1              | 0     |
|       | Placebo   | 0              | 0              | 1     |

Model is **overparameterized**SAS ignores the last level, making it the ref level

|    | Parameter      | Estimate    |   | Standard<br>Error | t Value | Pr >  t |
|----|----------------|-------------|---|-------------------|---------|---------|
| bo | Intercept      | 2.200000000 | В | 0.62716292        | 3.51    | 0.0043  |
| 01 | dose High Dose | 2.800000000 | В | 0.88694231        | 3.16    | 0.0083  |
| 02 | dose Low Dose  | 1.000000000 | В | 0.88694231        | 1.13    | 0.2816  |
| b3 | dose Placebo   | 0.000000000 | В | 8                 |         |         |





# **ANOVA** as Regression

$$libido_i = b_0 + b_1 X_1 + b_2 X_2 + e_i$$

 $X_1 = 1$  if observation belongs to the High Dose group and o otw

 $X_2 = 1$  if observation belongs to the Low Dose group and o otw

$$E(libido|Placebo) = b_0 = \bar{x}_{placebo}$$

$$E(libido|High) = b_0 + b_1 = \bar{x}_{high}$$

$$E(libido|Low) = b_0 + b_2 = \bar{x}_{low}$$

| Parameter      | Estimate    |   | Standard<br>Error | t Value | Pr >  t |
|----------------|-------------|---|-------------------|---------|---------|
| Intercept      | 2.200000000 | В | 0.62716292        | 3.51    | 0.0043  |
| dose High Dose | 2.800000000 | В | 0.88694231        | 3.16    | 0.0083  |
| dose Low Dose  | 1.000000000 | В | 0.88694231        | 1.13    | 0.2816  |
| dose Placebo   | 0.000000000 | В | 8                 |         |         |





#### Ismeans

- Least-squares means are predictions from a linear model
  - Balanced data: Ismeans = arithmetic group means
    - All SE are equal (due to homogeneity of variance assumption)
  - ▶ Unbalanced data: Ismeans ≠ arithmetic group means, adjusted for imbalance





# **Multiple Choice Poll**

 If you have 20 observations in your ANOVA and you calculate the residuals, to which of the following would they sum?

```
a. -20
```

b. o

C. 20

d. 400

e. Unable to tell from the information given





#### Multiple Choice Poll – Correct Answer

• If you have 20 observations in your ANOVA and you calculate the residuals, to which of the following would they sum?

- a. -20
- (b.) (
  - C. 20
  - d. 400
  - e. Unable to tell from the information given





# **Multiple Choice Poll**

- If you have 20 observations in your ANOVA and you calculate the squared residuals, to which of the following would they sum?
- a. -20
- b. o
- C. 20
- d. 400
- e. Unable to tell from the information given





### Multiple Choice Poll – Correct Answer

 If you have 20 observations in your ANOVA and you calculate the squared residuals, to which of the following would they sum?

- a. -20
- b. 0
- C. 20
- d. 400
- e. Unable to tell from the information given





# **Multiple Choice Poll**

- Which part of the ANOVA tables contains the variation due to nuisance factors?
- a. Sum of Squares Model
- b. Sum of Squares Error
- c. Degrees of Freedom





#### Multiple Choice Poll – Correct Answer

- Which part of the ANOVA tables contains the variation due to nuisance factors?
- a. Sum of Squares Model
- b.) Sum of Squares Error
  - c. Degrees of Freedom





## Multiple Answer Poll

 A study is conducted to compare the average monthly credit card spending for males versus females. Which statistical method might be used?

- a. One-sample *t*-test
- b. Two-sample *t*-test
- c. One-way ANOVA
- d. Two-way ANOVA





### Multiple Answer Poll – Correct Answers

• A study is conducted to compare the leaf area of leaf 4 of B73 versus Mo17. Which statistical method might be used?

a. One-sample *t*-test

b. Two-sample *t*-test

c. One-way ANOVA

d. Two-way ANOVA



