#### 431 Class 21

Thomas E. Love

2018-11-13

#### Today's Agenda

- Comparing 3 or more Population Means with the Analysis of Variance
- Indicator Variable Regression Analysis
- Interpreting the ANOVA table
- ANOVA assumptions and the Kruskal-Wallis test
- The Problem of Multiple Comparisons
  - Bonferroni pairwise testing
  - Tukey HSD pairwise comparisons
- Designing an ANOVA study: Power and Sample Size considerations

Almost all of this material is discussed in the Course Notes, mostly in Chapter 28.

#### Today's R Setup

```
source("Love-boost.R") # helps to load Hmisc explicitly
library(Hmisc); library(magrittr); library(broom)
library(readxl) # to read in an .xlsx file
library(tidyverse) # always load tidyverse last
```

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## County Health Rankings Data for Ohio, 2018

#### Data Source:

http://www.countyhealthrankings.org/app/ohio/2018/downloads

In the ohio\_2018.xlsx file I have provided to you, each row describes one of Ohio's 88 counties in terms of:

- FIPS code (basically an identifier for mapping)
- state and county name
- health outcomes (standardized more positive means better outcomes)
- health behavior ranking (1-88, we'll divide into 4 groups)
- clinical care ranking (1-88, we'll split into 3 groups)
- population density (urban or rural)
- median income, in dollars

```
ohio18 <- read xlsx("data/ohio 2018 rankings.xlsx") %>%
  mutate(behavior = cut2(rk behavior, g = 4),
         clin care = cut2(rk clin care, g = 3)) %>%
  mutate(behavior = fct recode(behavior,
            "Best" = "[ 1,23)", "High" = "[23,45)",
            "Low" = "[45,67)", "Worst" = "[67,88]")) %>%
  mutate(clin care = fct recode(clin care,
            "Strong" = "[ 1,31)", "Middle" = "[31,60)",
            "Weak" = "[60,88]")) %>%
  mutate(density = factor(density)) %>%
  select(FIPS, state, county, outcomes,
         behavior, clin_care, density, income)
```

```
ohio18 %>% filter(county == "Cuyahoga") %>%
 select(FIPS, county, outcomes, behavior, clin_care)
# A tibble: 1 x 5
 FIPS county outcomes behavior clin_care
 <chr> <chr> <chr> <dbl> <fct> <fct>
1 39035 Cuyahoga -0.38 Low Strong
ggplot(ohio18, aes(x = "", y = outcomes)) +
 geom_boxplot() + coord_flip() + labs(x = "")
                            outcomes
```

## Use inspect to inspect the data frame?

```
ohio18 %>% select(outcomes, behavior, clin_care) %>%
 mosaic::inspect()
categorical variables:
      name class levels n missing
1 behavior factor 4 88
2 clin care factor 3 88
                                 distribution
1 Best (25%), High (25%) ...
2 Strong (34.1%), Middle (33%) ...
quantitative variables:
     name class min Q1 median Q3
                                             max
1 outcomes numeric -1.88 -0.6125 0.015 0.6575 2.27
                     sd
                         n missing
          mean
```

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1 -0.0001136364 0.8940885 88

## **Key Measure Details**

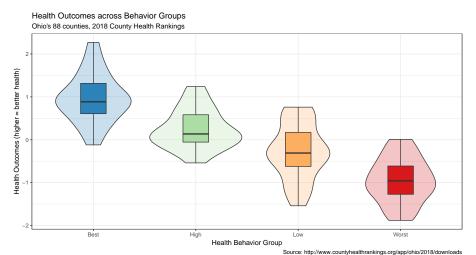
- **outcomes** = quantity that describes the county's premature death and quality of life results, weighted equally and standardized (z scores).
  - Higher (more positive) values indicate better outcomes in this county.
- behavior = (Best/High/Low/Worst) reflecting adult smoking, obesity, food environment, inactivity, exercise, drinking, alcohol-related driving deaths, sexually tranmitted infections and teen births.
  - Counties in the Best group had the best behavior results.
- clin\_care = (Strong/Middle/Weak) reflects rates of uninsured, care providers, preventable hospital stays, diabetes monitoring and mammography screening.
  - Strong means that clinical care is strong in this county.

#### **Our Questions**

- O Do average health outcomes vary significantly across groups of counties defined by health behavior?
- ② Do groups of counties defined by clinical care show meaningful differences in average health outcomes?

#### Question 1

#### Do average health outcomes differ by health behavior?



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#### **Question 1 Numerical Summaries**

Do average health outcomes vary significantly across groups of counties defined by health behavior?

```
mosaic::favstats(outcomes ~ behavior, data = ohio18) %>%
knitr::kable(digits = 2)
```

behavior	min	Q1	median	Q3	max	mean	sd	n	missing
Best	-0.12	0.61	0.88	1.31	2.27	0.97	0.57	22	(
High	-0.54	-0.06	0.14	0.58	1.24	0.25	0.45	22	(
Low	-1.54	-0.62	-0.31	0.17	0.76	-0.28	0.65	22	(
Worst	-1.88	-1.27	-0.96	-0.61	0.01	-0.95	0.52	22	(

Note that there is no missing data here.

## Analysis of Variance (ANOVA) testing: Question 1

Does the mean outcomes result differ across the behavior groups?

 $H_0: \mu_{Best} = \mu_{High} = \mu_{Low} = \mu_{Worst}$  vs.  $H_A:$  At least one  $\mu$  is different.

To test this set of hypotheses, we will build a linear model to predict each county's outcome based on what behavior group the county is in.

- We then look at whether the behavior group effect has a statistically significant impact on the model's predictions of outcomes.
- If behavior has a significant effect in that model, it means that we reject  $H_0$  in favor of  $H_A$ .

### **Building the Linear Model: Question 1**

Are there statistically significant differences in mean outcome across the behavior group means?

```
model_one <- lm(outcomes ~ behavior, data = ohio18)
model_one</pre>
```

```
Call:
lm(formula = outcomes ~ behavior, data = ohio18)

Coefficients:
(Intercept) behaviorHigh behaviorLow
0.9718 -0.7186 -1.2495

behaviorWorst
-1.9195
```

How do we interpret this model?

## Interpreting the Indicator Variables

The regression model (model\_one) equation is

outcomes = 0.97 - 0.72 behaviorHigh
- 1.25 behaviorLow
- 1.92 behaviorWorst

What do the indicator variables mean?

group	behaviorHigh	behaviorLow	behaviorWorst
Best	0	0	0
High	1	0	0
Low	0	1	0
Worst	0	0	1

• So what is the predicted outcomes score for a county in the High behavior group, according to this model?

### Interpreting the Indicator Variables

The regression model (model\_one) equation is

outcomes = 0.97 - 0.72 behaviorHigh
- 1.25 behaviorLow
- 1.92 behaviorWorst

What predictions does the model make?

group	High	Low	Worst	Prediction
Best	0	0	0	0.97
High	1	0	0	0.97 - 0.72 = 0.25
Low	0	1	0	0.97 - 1.25 = -0.28
Worst	0	0	1	0.97 - 1.92 = -0.95

Do these predictions make sense?

```
The regression model (model_one) equation is

outcomes = 0.97 - 0.72 behaviorHigh

- 1.25 behaviorLow

- 1.92 behaviorWorst
```

Recall that the sample data shows...

```
ohio18 %>% group_by(behavior) %>%
summarize(n = n(), mean = round(mean(outcomes),2))
```

```
# A tibble: 4 x 3
behavior n mean
<fct> <int> <int> <0000
1 Best 22 0.97
2 High 22 0.25
3 Low 22 -0.28
4 Worst 22 -0.95
```

#### ANOVA for the Linear Model: Question 1

Are there statistically significant differences in mean outcome across the behavior group means?

 $H_0$ :  $\mu_{Best} = \mu_{High} = \mu_{Low} = \mu_{Worst}$  vs.  $H_A$ : At least one  $\mu$  is different.

```
anova(model_one)
```

Analysis of Variance Table

```
Response: outcomes

Df Sum Sq Mean Sq F value Pr(>F)
behavior 3 43.645 14.5482 47.179 < 2.2e-16 ***
Residuals 84 25.903 0.3084
---
Signif. codes:
0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

## So, what's in the ANOVA table? (df)

The ANOVA table reports here on a single **factor** (behavior group) with 3 levels, and on the residual variation in health **outcomes** not accounted for by that factor.

```
anova(model_one)[1:4]
```

```
Df Sum Sq Mean Sq F value
behavior 3 43.645 14.5482 47.179
Residuals 84 25.903 0.3084
```

**Degrees of Freedom** (df) is an index of sample size. . .

- df for our factor (behavior) is one less than the number of categories. We have four behavior groups, so 3 degrees of freedom.
- Adding df(behavior) + df(Residuals) = 3 + 84 = 87 = df(Total), one less than the number of observations (counties) in Ohio.
- n observations and g groups yield n-g-1 residual df in a one-factor ANOVA table.

## So, what's in the ANOVA table? (Sum of Squares)

anova(model\_one)[1:4]

Df Sum Sq Mean Sq F value behavior 3 43.645 14.5482 47.179 Residuals 84 25.903 0.3084

Sum of Squares (Sum Sq, or SS) is an index of variation...

- SS(factor), here SS(behavior) measures the amount of variation accounted for by the behavior groups in our model\_one.
- The total variation in outcomes to be explained by the model is SS(factor) + SS(Residuals) = SS(Total) in a one-factor ANOVA table.
- We describe the proportion of variation explained by a one-factor ANOVA model with  $\eta^2$  ("eta-squared": same as Multiple  $R^2$ )

$$\eta^2 = \frac{SS(\text{behavior})}{SS(\text{Total})} = \frac{43.645}{43.645 + 25.903} = \frac{43.645}{69.548} \approx 0.628$$

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## So, what's in the ANOVA table? (MS and F)

Df Sum Sq Mean Sq F value behavior 3 43.645 14.5482 47.179 Residuals 84 25.903 0.3084

 $\textbf{Mean Square} \; (\texttt{Mean Sq.} \; \mathsf{or} \; \mathsf{MS}) = \mathsf{Sum} \; \mathsf{of} \; \mathsf{Squares} \; / \; \mathsf{df}$ 

$$MS({
m behavior}) = rac{SS({
m behavior})}{df({
m behavior})} = rac{43.645}{3} pprox 14.55$$

- MS(Residuals) estimates the residual variance, the square of the residual standard deviation (residual standard error in earlier work).
- The ratio of MS values is the ANOVA F value.

ANOVA 
$$F = \frac{MS(\text{behavior})}{MS(\text{Residuals})} = \frac{14.5482}{0.3084} \approx 47.18$$

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## So, what's in the ANOVA table? (p value)

- The p value is derived from the ANOVA F statistic, as compared to the F distribution.
- Which F distribution is specified by the two degrees of freedom values, as the F table is indexed by both a numerator and a denominator df.

```
pf(47.17879, df1 = 3, df2 = 84, lower.tail = FALSE)
```

[1] 5.680062e-18

#### We could also have used...

Are there statistically significant differences in mean outcome across the behavior group means?

 $H_0: \mu_{Best} = \mu_{High} = \mu_{Low} = \mu_{Worst}$  vs.  $H_A:$  At least one  $\mu$  is different.

```
Df Sum Sq Mean Sq F value Pr(>F)
behavior 3 43.64 14.548 47.18 <2e-16 ***
Residuals 84 25.90 0.308
---
Signif. codes:
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

So, what's the conclusion? Is this a surprise?

#### Another identical approach

Are there statistically significant differences in mean outcome across the behavior group means?

 $H_0: \mu_{Best} = \mu_{High} = \mu_{Low} = \mu_{Worst}$  vs.  $H_A:$  At least one  $\mu$  is different.

One-way analysis of means

```
data: outcomes and behavior
F = 47.179, num df = 3, denom df = 84, p-value < 2.2e-16</pre>
```

### **ANOVA Assumptions**

The assumptions behind analysis of variance are the same as those behind a linear model. Of specific interest are:

- The samples obtained from each group are independent.
- Ideally, the samples from each group are a random sample from the population described by that group.
- In the population, the variance of the outcome in each group is equal. (This is less of an issue if our study involves a balanced design.)
- In the population, we have Normal distributions of the outcome in each group.

Happily, the ANOVA F test is fairly robust to violations of the Normality assumption.

# Is there an approach that doesn't assume equal variances?

Yes, but this isn't exciting if we have a balanced design.

```
oneway.test(outcomes ~ behavior, data = ohio18)
```

One-way analysis of means (not assuming equal variances)

```
data: outcomes and behavior F = 47.322, num df = 3.000, denom df = 46.314, p-value = 3.788e-14
```

 Note that this approach uses a fractional degrees of freedom calculation in the denominator.

#### The Kruskal-Wallis Test

If you thought the data were severely skewed, you might avoid the ANOVA and instead try:

```
kruskal.test(outcomes ~ behavior, data = ohio18)
```

Kruskal-Wallis rank sum test

```
data: outcomes by behavior
Kruskal-Wallis chi-squared = 57.049, df = 3,
p-value = 2.508e-12
```

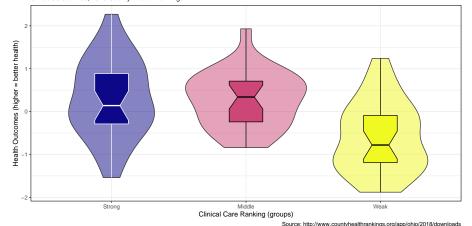
- H<sub>0</sub>: The four behavior groups have the same center to their outcomes distributions.
- $H_A$ : At least one group has a shifted distribution, with a different center to its outcomes.

What would be the conclusion in this case?

#### Question 2

Do groups of counties defined by clinical care show meaningful differences in average health outcomes?

Health Outcomes across County Clinical Care Ranking
Ohio's 88 counties, 2018 County Health Rankings



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#### **Question 2 Numerical Summaries**

Do groups of counties defined by clinical care show meaningful differences in average health outcomes?

```
mosaic::favstats(outcomes ~ clin_care, data = ohio18) %>%
  knitr::kable(digits = 2)
```

clin_care	min	Q1	median	Q3	max	mean	sd	n	missin
Strong	-1.54	-0.29	0.14	0.89	2.27	0.30	0.91	30	
Middle	-0.84	-0.24	0.34	0.71	1.93	0.28	0.65	29	
Weak	-1.88	-1.19	-0.78	-0.09	1.24	-0.59	0.82	29	

Trust me - there's no missing data here. Sorry the table cuts off.

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```
model2 <- lm(outcomes ~ clin care, data = ohio18)
anova(model2)
Analysis of Variance Table
Response: outcomes
          Df Sum Sq Mean Sq F value Pr(>F)
clin_care 2 15.221 7.6103 11.907 2.762e-05 ***
Residuals 85 54.327 0.6391
Signif. codes:
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
kruskal.test(outcomes ~ clin_care, data = ohio18)
```

Kruskal-Wallis rank sum test

```
data: outcomes by clin_care
Kruskal-Wallis chi-squared = 18.54, df = 2,
p-value = 9.422e-05
```

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## K-Sample Study Design, Comparing Means

- What is the outcome under study?
- ② What are the (in this case, K > 2) treatment/exposure groups?
- Were the data in fact collected using independent samples?
- Are the data random samples from the population(s) of interest? Or is there at least a reasonable argument for generalizing from the samples to the population(s)?
- What is the significance level (or, the confidence level) we require?
- Are we doing one-sided or two-sided testing? (usually 2-sided)
- What does the distribution of each individual sample tell us about which inferential procedure to use?
- Are there statistically meaningful differences between population means?
- If an overall test is significant, can we identify pairwise comparisons of means that show significant differences using an appropriate procedure that protects against Type I error expansion due to multiple comparisons?

### What's Left to do? (Multiple Comparisons)

If an overall test is significant, can we identify pairwise comparisons of means that show significant differences using an appropriate procedure that protects against Type I error expansion due to multiple comparisons?

Yes. There are two methods we'll study to identify specific pairs of means where we have statistically significant differences, while dealing with the problem of multiple comparisons.

- Bonferroni pairwise comparisons
- Tukey's HSD (Honestly Significant Differences) approach

# We found a significant difference between behavior groups

But which ones are different from which? All the ANOVA tells is that there is strong evidence that they aren't all the same.

```
anova(lm(outcomes ~ behavior, data = ohio18))
```

Analysis of Variance Table

```
Response: outcomes

Df Sum Sq Mean Sq F value Pr(>F)
behavior 3 43.645 14.5482 47.179 < 2.2e-16 ***
Residuals 84 25.903 0.3084
---
Signif. codes:
0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

Is, for example, Best significantly different from Worst?

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#### Could we just run a bunch of t tests?

This approach assumes that you need to make no adjustment for the fact that you are doing multiple comparisons, simultaneously.

Pairwise comparisons using t tests with pooled SD

data: ohio18\$outcomes and ohio18\$behavior

```
Best High Low
High 4.7e-05 - -
Low 7.1e-11 0.00212 -
Worst < 2e-16 2.7e-10 0.00013
```

P value adjustment method: none

### The problem of Multiple Comparisons

 The more comparisons you do simultaneously, the more likely you are to make an error.

In the worst case scenario, suppose you do two tests - first A vs. B and then A vs. C, each at the  $\alpha=0.10$  level.

• What is the combined error rate across those two t tests?

### The problem of Multiple Comparisons

In the worst case scenario, suppose you do two tests - first A vs. B and then A vs. C, each at the  $\alpha=0.10$  level.

• What is the combined error rate across those two t tests?

Run the first test. Make a Type I error 10% of the time.

A vs B Type I error	Probability
Yes	0.1
No	0.9

Now, run the second test. Assume (perhaps wrongly) that comparing A to C is independent of your A-B test result. What is the error rate now?

## The problem of Multiple Comparisons

In the worst case scenario, suppose you do two tests - first A vs. B and then A vs. C, each at the  $\alpha=0.10$  level.

• What is the combined error rate across those two t tests?

Assuming there is a 10% chance of making an error in either test, independently . . .

_	Error in A vs. C	No Error	Total
Type I error in A vs. B	0.01	0.09	0.10
No Type I error in A-B	0.09	0.81	0.90
Total	0.10	0.90	1.00

So you will make an error in the A-B or A-C comparison 19% of the time, rather than the nominal  $\alpha = 0.10$  error rate.

#### But in our case, we're building SIX tests

- Best vs. High
- Best vs. Low
- Best vs. Worst
- 4 High vs. Low
- 6 High vs. Worst
- 6 Low vs. Worst

and if they were independent, and each done at a 5% error rate, we could still wind up with an error rate of

$$.05 + (.95)(.05) + (.95)(.95)(.05) + (.95)^3(.05) + (.95)^4(.05) + (.95)^5(.05)$$
  
= .265

Or worse, if they're not independent.

#### The Bonferroni Method

If we do 6 tests, we could just reduce the necessary  $\alpha$  to 0.05 / 6 = 0.0083 and that would maintain an error rate no higher than  $\alpha$  = 0.05 across those tests.

• Or we could let R adjust the p values directly. . .

Pairwise comparisons using t tests with pooled SD

data: ohio18\$outcomes and ohio18\$behavior

```
Best High Low
High 0.00028 - -
Low 4.3e-10 0.01273 -
Worst < 2e-16 1.6e-09 0.00081
```

## **Tukey Honestly Significant Differences (HSD)**

Tukey's HSD approach is a better choice for pre-planned comparisons with a balanced (or nearly balanced) design. It provides confidence intervals and an adjusted p value for each comparison.

• Let's run some confidence intervals to yield an overall 99% confidence level, even with 6 tests...

Output on the next slide...

Tukey multiple comparisons of means 99% family-wise confidence level factor levels have been ordered

Fit: aov(formula = lm(outcomes ~ behavior, data = ohio18))

#### \$behavior

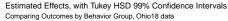
difflwruprp adjLow-Worst0.67000000.1326937361.2073060.0007665High-Worst1.20090910.6636028271.7382150.0000000Best-Worst1.91954551.3822391902.4568520.0000000High-Low0.5309091-0.0063971731.0682150.0111954Best-Low1.24954550.7122391901.7868520.0000000Best-High0.71863640.1813300991.2559430.0002716

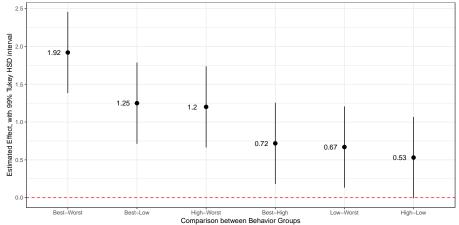
#### Tidying the Tukey HSD confidence intervals

term	comparison	estimate	conf.low	conf.high	adj.p.value
behavior	Low-Worst	0.670	0.133	1.207	0.001
behavior	High-Worst	1.201	0.664	1.738	0.000
behavior	Best-Worst	1.920	1.382	2.457	0.000
behavior	High-Low	0.531	-0.006	1.068	0.011
behavior	Best-Low	1.250	0.712	1.787	0.000
behavior	Best-High	0.719	0.181	1.256	0.000

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#### Plotting Your Tukey HSD intervals, Approach 1





```
ggplot(tukey_one, aes(x = reorder(comparison, -estimate),
                      y = estimate)) +
  geom_pointrange(aes(ymin = conf.low, ymax = conf.high)) +
  geom_hline(yintercept = 0, col = "red",
             linetype = "dashed") +
  geom_text(aes(label = round(estimate,2)), nudge_x = -0.2) +
  theme bw() +
  labs(x = "Comparison between Behavior Groups",
       y = "Estimated Effect, with 99% Tukey HSD interval",
       title = "Estimated Effects, with Tukey HSD 99% Confiden
       subtitle = "Comparing Outcomes by Behavior Group, Ohio:
```

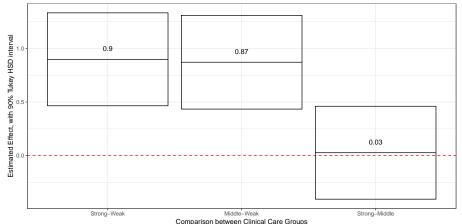
## Question 2: 90% Tukey HSD intervals, tidying

term	comparison	estimate	conf.low	conf.high	adj.p.value
clin_care	Middle-Weak	0.871	0.434	1.307	0.000
clin_care	Strong-Weak	0.898	0.465	1.331	0.000
clin_care	Strong-Middle	0.027	-0.406	0.460	0.991

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# Plotting Question 2 Tukey HSD intervals

Estimated Effects, with Tukey HSD 90% Confidence Intervals Comparing Outcomes by Clinical Care Group, Ohio18 data



Comparison between Clinical Care Groups

```
ggplot(tukey two, aes(x = reorder(comparison, -estimate),
                      y = estimate)) +
  geom crossbar(aes(ymin = conf.low, ymax = conf.high),
                fatten = 1) +
  geom hline(yintercept = 0, col = "red",
             linetype = "dashed") +
  geom_text(aes(label = round(estimate,2)), nudge_y = 0.1) +
  theme bw() +
  labs(x = "Comparison between Clinical Care Groups",
       y = "Estimated Effect, with 90% Tukey HSD interval",
       title = "Estimated Effects, with Tukey HSD 90% Confiden
       subtitle = "Comparing Outcomes by Clinical Care Group,
```

# Power/Sample Size for designing an ANOVA study

Is there a power.anova.test approach in R? Sure.

- groups = number of groups
- n = number of observations per group
- between.var = between-group variance
- within.var = within-group variance
- sig.level =  $\alpha$  (significance level)
- power =  $1 \beta$

Specify five, and the computer will calculate the sixth. This does require a **balanced design**.

So, what do we use for between.var and within.var?

#### Determining between.var and within.var

- If you have prior knowledge of what you expect the true group means to be, then you can take their variance to get the between.var value.
- The within.var value is the within-group variance. To get that, realize that ANOVA assumes that each group will have the same standard deviation of outcome values. Square that "within-group standard deviation" you estimate to obtain the within-group variance.

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# Powering an ANOVA study, Setup

#### PI wants to plan a study:

- to compare four groups, and she wants to be sure she can detect a difference if the means turn out to be any more different than they would be if they were 560, 585, 610 and 625.
- ullet using a balanced design, 90% power and lpha=0.05
- where she thinks that the standard deviation in each group of the scores will be 80.

power.anova.test needs five of these six things:

- groups = number of groups
- n = number of observations per group
- between.var = between-group variance
- within.var = within-group variance
- sig.level =  $\alpha$  (significance level)
- power =  $1 \beta$

Balanced one-way analysis of variance power calculation

```
groups = 4

n = 38.01195

between.var = 816.6667

within.var = 6400

sig.level = 0.05

power = 0.9
```

NOTE: n is number in each group

# Is there a power and sample size approach for unbalanced ANOVA?

Not a simple one, and not within the pwr package, no.

#### **Next Time**

- Multiple Regression: The Fundamentals
- Project Study 2: Demonstration