BMEG 802 – Advanced Biomedical Experimental Design and Analysis

Analysis of Coariance (ANCOVA)

Joshua G. A. Cashaback, PhD

Recap

- Mixed (Between-Within) Design
  - 2 or more groups
  - 2 or more repeated measures

Today

- Analysis of Covariance
  - Similar to ANOVA
  - But controlling for covariates (e.g., age)
    - Regress out covariates
  - Can do for between and within designs
    - We'll go over a between example today

Concomitant Variables (covariates)

- A variable that has been collected for each subject in advance (usually) of the experimental manipulation
- e.g. Pre-existing differences among subjects on some (continuous) variable

Two Ways to Use Covariates

1. covariates can be used **BEFORE** the experiment to experimentally control for pre-existing differences

- use score on a covariate as a way to assign subjects to groups
- e.g. match different groups based on score on a covariate measure
- e.g. match different treatment groups on age, or gender, or IQ
  - 'age-matched controls'
- Good experimental design is important!
  - But, sometimes this is difficult / not possible.

Two Ways to Use Covariates

2. covariates can be used **AFTER** the experiment to “statistically” adjust for pre-existing differences

- allow for variation in the concomitant variable both within and between groups
- during the analysis, statistically account for the effect of the covariate on the dependent variable

ANCOVA

- ANCOVA is a method to account for the relationship (if it exists) between a covariate and a dependent variable
- It is a way to statistically adjust for differences on the concomitant variable by including it as a predictor variable
- ANCOVA is just like regular ANOVA in terms of the model comparison approach
  - we have a restricted model and a full model
  - note however one of the variables in our model (i.e. the covariate) is a continuous variable

Logic of ANCOVA

- Would the groups have been different on the dependent variable, if they had been equivalent on the covariate?
  - i.e. is the observed difference in the dependent variable due to our experimental manipulation, or can it be explained by the pre-existing differences in the covariate?

Inclusion of the Covariate

Including a covariate in the model affects the analysis in two ways:

1. Within-group variability will be reduced
   - by an amount dependent on the strength of the linear relationship between the dependent variable and the covariate
   - typically a substantial reduction in unexplained variance
   - a smaller error term
   - greater power
2. An increase in the estimate of the effect size

NOT a substitution for randomization

- ANCOVA will only equate groups “statistically” on a single covarying variable
- randomization (over the long run) guarantees that groups will be equated on ALL relevant dimensions, not just the covariate

Examples of an ANCOVA

1. Want to test the effects of a drug, but controlling for expected effects of age
   - age is the covariate
2. Testing the effects of learning but controlling for individual differences in performance
   - pre-test score is the covariate
3. Determine whether a new exercise leads to better performance but control from VO2 max.
   - V02 max is the covariate

ANCOVA

- Same concepts, assumptions and principles apply as other within and / or between ANOVA
  - Linear model
  - Assumptions + some ANCOVA specific ones
    - Homogeneity of Regression
  - Interactions, main effects
    - Follow-up ADJUSTED mean comparisons
  - Except now we are controlling for covariates

General Linear Model (GLM)

Full Model: $Y_{ij} = \mu + \alpha_j + \beta_{ij} + \epsilon_{ijk}$

Restricted Model: $Y_{ij} = \mu + \beta_{ij} + \epsilon_{ijk}$

Effect of Between Factor

Covariate - β_{ij} is the regression coefficient

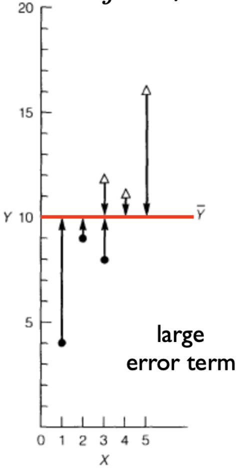
- estimation is done using a least-squares criterion
- same thing as a linear regression
- cost $=$ we lose a single df
- β is an extra variable in our model

see Maxwell, Delaney, Kelley for details (error, df, etc.)

Graphing the Covariate

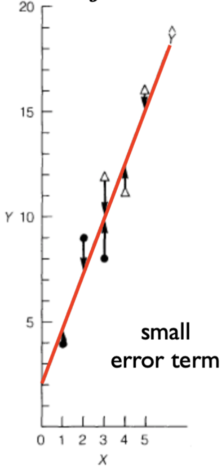
ANOVA

$$Y_{ij} = \mu + \epsilon_{ij}$$



ANCOVA

$$Y_{ij} = \mu + \boxed{\beta X_{ij}} + \epsilon_{ij}$$

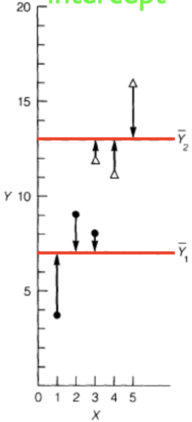


Graphing Effect of Group With and Without Covariate

ANOVA

$$Y_{ij} = \mu + \alpha_j + \epsilon_{ij}$$

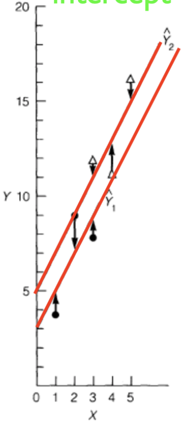
intercept



ANCOVA

$$Y_{ij} = \mu + \alpha_j + \beta X_{ij} + \epsilon_{ij}$$

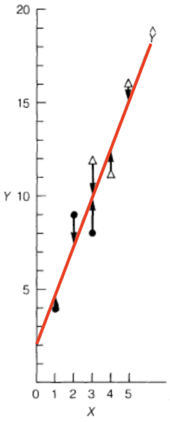
intercept slope



Graphing Restricted vs. Full Model

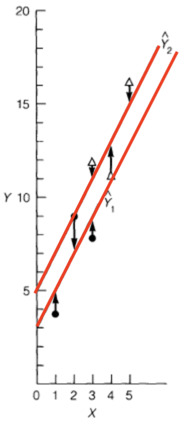
restricted model

$$Y_{ij} = \mu + \beta X_{ij} + \epsilon_{ij}$$



full model

$$Y_{ij} = \mu + \alpha_j + \beta X_{ij} + \epsilon_{ij}$$



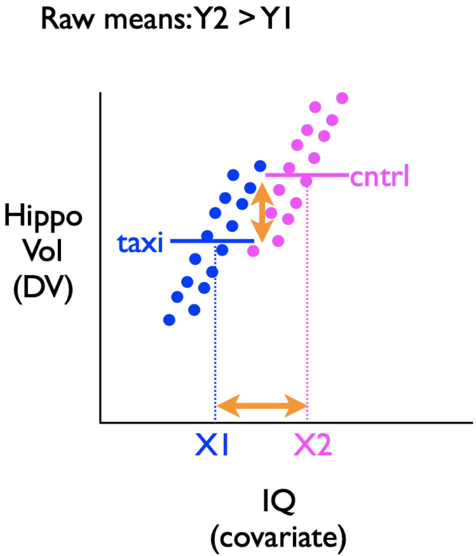
Adjusted (corrected) Means

- once you account for the relationship between a covariate and the dependent variable, you can generate “adjusted means” for each group
- What would the means of each group be, if the scores on the covariate had been equal?
- Adjust mean of each group in proportion to the linear relationship between covariate and DV
- Let’s think about this use a fictitious example.

Adjusted (‘Corrected’) Means with Example

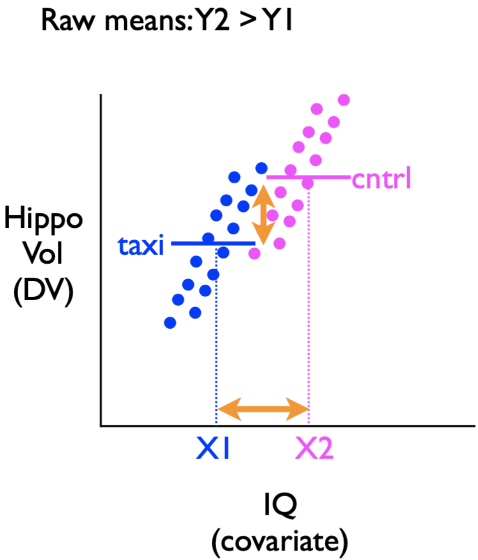
- the hippocampus is a brain structure thought to be involved in spatial memory
- is the volume of the hippocampus greater for taxi drivers than for people who aren’t taxi drivers?
- 2 groups of people (taxi drivers, non-taxi drivers) are scanned using MRI, & volume of hippocampus is estimated
- problem: let’s say we know that hippocampus volume is also related to general intelligence*
- we can include IQ as a covariate in the analysis

Raw Means



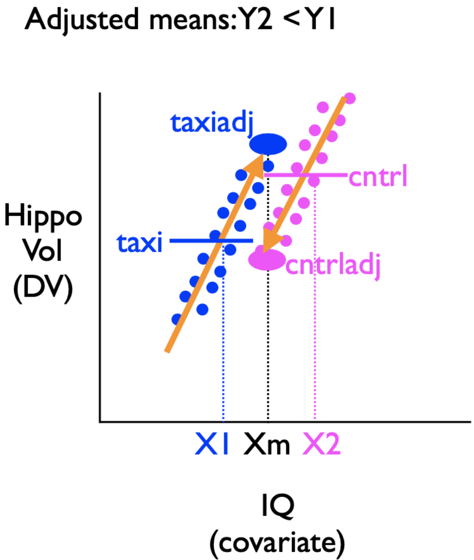
- HippoVol of group 1 (taxiDrvrs) is less than group 2 (controls)

But What About the Covariate?



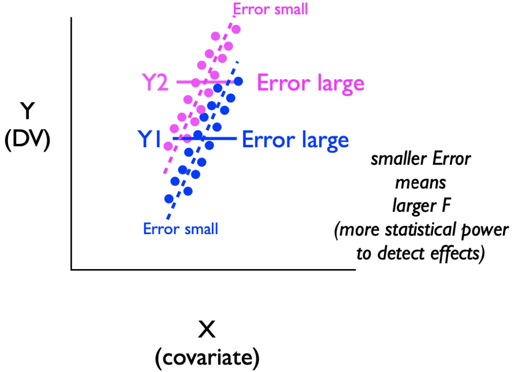
- HippoVol of group 1 (taxiDrvrs) is less than group 2 (controls)
- oops!
- but it happens that our controls tended to have higher IQ
- what if we "statistically" control for these pre-existing IQ differences?

Adjusted Means



- let's use the known relationship between IQ (X) and HippoVol (Y) to predict,
- what would HippoVol (Y) have been,
- IF IQ of both groups were equal?
- IF they were equal to (for example) the grand mean of X (IQ)
- now we see that on the adjusted means, HippoVol for taxi drivers is > than controls

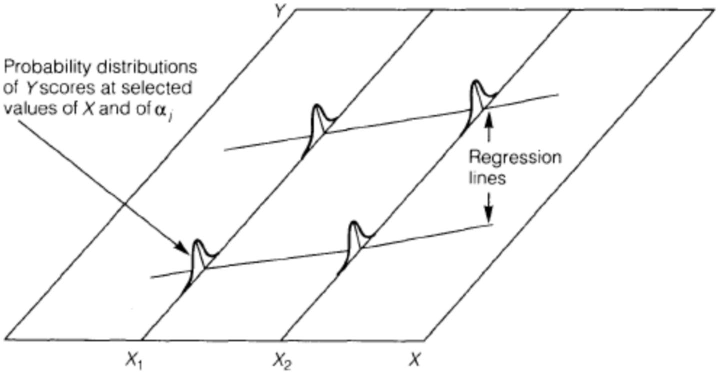
Adjusted Means Cont'd



ANCOVA can help even if there are no pre-existing group differences on the covariate

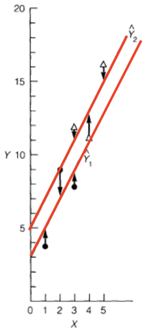
ANCOVA Assumptions - Normality

- DV scores must be normally distributed
  - for all values of the covariates



ANCOVA Assumptions - Homogeneity of Regression

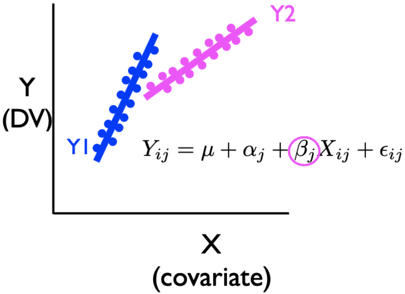
- separate within-group regression lines have the same slope
- there is only one Beta coefficient
  - (no subscript j for β)



Although. . . .

ANCOVA Assumptions - Homogeneity of Regression

- You can have different covariate slopes
- BUT, you lose one df for every j (e.g., β_j)



- Yet still, if Y1 and Y2 overlap in X, but they have different slopes, what does that mean?
- Suggestion, stick with a single β

Further ANCOVA Assumptions

- Linear Relationship Between DV and Covariate
- Independence between covariate and treatment
  - e.g., what if an experimental treatment (e.g. a drug) actually has different effects on young vs old people?
  - we can't tell; statistically adjusting for the means on the covariate won't solve this problem
  - the only way to truly deal with this is to equate groups on age in the first place
    - again, a good statistical design is best.

Pre-Post Designs vs ANCOVA

- why not just do an ANOVA on the difference scores between pre- and post?
  - we can control for individual differences.
- slope of covariate is assumed to be 1.0
- when slope of relationship between covariate and DV is not one, this is a less sensitive model (bigger error term) than one where you can estimate the slope (even though you give up a df for estimating the slope)

ANCOVA Example in R

Let's determine if there is a main effect of Class Section (I vs II), which is taught by different instructors, on calculus grades. We have there average in their respective calculus section. But we also want to control for their previous high school marks to make sure students that typically score better were not randomly placed in the same section. Are one of the instructors better than the other?

- First, lets see what would happen if we did a 1-way ANOVA without considering the covariate.
- Second, lets control for the covariate by performing an ANCOVA.

Subject	Group	Calculus Grade	High School Grade
1	I	10	20
2	I	30	45
3	I	50	40
4	II	60	60
5	II	80	55
6	II	100	80

Setting up the Data

```r
calculus <- c(10,30,50,60,80,100)
highschool <- c(20,45,40,60,55,80)
section <- factor(c(rep('1',3),rep('2',3)))
subject <- factor(c(1,2,3,4,5,6))
mydata <- data.frame(subject, section, calculus, highschool)
```

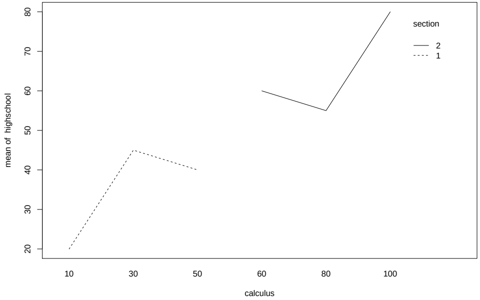
Setting up the Data

```
mydata
```

```
##   subject section calculus highschool
## 1       1       1       10         20
## 2       2       1       30         45
## 3       3       1       50         40
## 4       4       2       60         60
## 5       5       2       80         55
## 6       6       2      100         80
```

Plot Data

```
interaction.plot(calculus, section, highschool)
```



Install Mixed ANOVA Package

install.package(“rstatix”)

```
library(rstatix)
```

```
##
## Attaching package: 'rstatix'

## The following object is masked from 'package:stats':
##
##     filter
```

ANOVA (no covariates)

```
# note, we are not considering high school marks
res.aov <- anova_test(calculus ~ section, data = mydata, type = 3, effect.s
```

```
## Coefficient covariances computed by hccm()
```

```
res.aov
```

```
## ANOVA Table (type III tests)
##
##    Effect DFn DFd     F     p p<.05   pes
## 1 section   1   4 9.375 0.038     * 0.701
```

Here we might (falsely) conclude that the Section II instructor is better (because we didn't consider covariates)

ANCOVA (controlling for covariates)

```
# note, we are controlling for high school marks
res.aov <- anova_test(calculus ~ section + highschool, data = mydata,
                      type = 3, effect.size = "pes")
```

```
## Coefficient covariances computed by hccm()
```

```
res.aov
```

```
## ANOVA Table (type III tests)
##
##       Effect DFn DFd     F     p p<.05   pes
## 1    section   1   3 0.553 0.511       0.156
## 2 highschool   1   3 4.000 0.139       0.571
```

When controlling for previous performance in highschool, these results suggest that the instructors in section I and II are similar [F(1,3) = 0.553, p = 0.511]

ANCOVA - Linearity Assumption

There should be a significantly linear relationship between the dependent variable and covariate linear for each subgroup

```r
cor.test(calculus[1:3],highschool[1:3])$p.value
```

```
## [1] 0.4543711
```

```r
cor.test(calculus[4:6],highschool[4:6])$p.value
```

```
## [1] 0.4543711
```

These are greater than 0.05, thus there isn't a significant relationship

- Technically, we shouldn't be using ANCOVA in this situation
- We are probably under-powered (3 per group)
- But lets continue the example.

ANCOVA - Homogeneity of Regression Slopes

Assume that the regression lines are co-linear. We can check for this by checking whether there is a significant interaction between the grouping variable (e.g., section) and the covariate

```
anova_test(calculus ~ section*highschool, data = mydata)
```

```
## Coefficient covariances computed by hccm()

## ANOVA Table (type II tests)
##
##               Effect DFn DFd     F     p p<.05   ges
## 1            section   1   2 0.369 0.605       0.156
## 2         highschool   1   2 2.667 0.244       0.571
## 3 section:highschool   1   2 0.000 1.000       0.000
```

There is homogeneity of regression slopes as there was not a significant interaction between Section and high school marks [F(1,2) = 0.000, p = 1.000)]

ANCOVA - Normality of Residuals

```
# fit the linear model (covariate goes first)
model <- lm(calculus ~ highschool + section, data = mydata)
# find residuals of the linear model fit
model.metrics <- augment(model)
# test whether residulas are normally distributed
shapiro_test(model.metrics$.resid)
```

```
## # A tibble: 1 x 3
##   variable              statistic p.value
##   <chr>                     <dbl>   <dbl>
## 1 model.metrics$.resid      0.933   0.602
```

The Shapiro Wilk test was not significant ($p > 0.05$), so we can assume normality of residuals

ANCOVA - Homogeneity of Variance

Normality of Residuals

```
model.metrics %>% levene_test(.resid ~ section)
```

```
## # A tibble: 1 x 4
##     df1   df2 statistic     p
##   <int> <int>     <dbl> <dbl>
## 1     1     4  1.07e-32     1
```

The Levene's test was not significant ($p > 0.05$), so we can assume homogeneity of the residual variances for all groups.

ADJUSTED Mean Comparisons

Let's compare the Adjusted Means

```
library(emmeans)
pwc <- emmeans_test(mydata, calculus ~ section, covariate = highschool, p.adjust.method = "holm")
pwc
```

```
## # A tibble: 1 x 9
##   term            .y.     group1 group2    df statistic     p p.adj p.adj.signif
## * <chr>           <chr>   <chr>  <chr>  <dbl>     <dbl> <dbl> <dbl> <chr>
## 1 highschool*sec~ calcul~ 1      2          3    -0.744 0.511 0.511 ns
```

```
get_emmeans(pwc)
```

```
## # A tibble: 2 x 8
##   highschool section emmean    se    df conf.low conf.high method
##        <dbl> <fct>    <dbl> <dbl> <dbl>    <dbl>     <dbl> <chr>
## 1         50 1         47.1  12.2     3     8.21      86.1 Emmeans test
## 2         50 2         62.9  12.2     3    23.9      102.  Emmeans test
```

Adjusted means are not significantly different ($p = 0.511$)

Outputting Adjusted Means

```r
em_section <- emmeans(model, ~section)
em_section
```

```
##  section emmean   SE df lower.CL upper.CL
##  1         47.1 12.2  3     8.21     86.1
##  2         62.9 12.2  3    23.92    101.8
##
## Confidence level used: 0.95
```

Adjusted means are 57.1 and 62.9

Outputting Sample Effect Size

Let's quickly split our data by

```
d = eff_size(em_section, sigma = sigma(model), edf = df.residual(model))
d
```

```
##  contrast effect.size   SE df lower.CL upper.CL
##  1 - 2          -1.04 1.46  3    -5.69     3.61
##
## sigma used for effect sizes: 15.12
## Confidence level used: 0.95
```

Cohen's d = 1.04

Mixed ANOVA Interpretation

- There was not a significant effect of main effect of class section on calculus grades when accounting for the covariate of high school grades [$F(1,3) = 0.553$, $p = 0.511$, $\eta_p^2 = 0.156$]. Thus there are no significant differences between the two instructors.
- Note, same rules apply on the order to examine interaction or main effects as in 2-way between ANOVA and 2-way within ANOVA designs.

Power Analysis on ANCOVA

install.packages("WebPower")

```
library(WebPower)
```

```
## Loading required package: MASS

##

## Attaching package: 'MASS'

## The following object is masked from 'package:rstatix':
##
##     select

## Loading required package: lme4

## Loading required package: Matrix

## Registered S3 methods overwritten by 'lme4':
##   method                          from
##   cooks.distance.influence.merMod car
```

Power Analysis on ANCOVA

install.packages("pwr2ppl")

```
library(pwr2ppl)
# m1.1, s1.1 = mean and stdev for group 1.
# r = correlation between dv and covariate
anc(m1.1=.85,m2.1=2.5, s1.1 = 1.7, s2.1=1, alpha = 0.05,
    r= 0.4, n=12, factors = 1,levelsA = 2)
```

```
## Sample size per cell = 12

## Power IV1 = 0.8335 for partial eta-squared = 0.31
```

We need 12 participants per group for a sufficiently powered.

- Note had to iterate through n to find power over 80%
- For more information on other ANCOVA designs: https://cran.r-project.org/web/packages/pwr2ppl/pwr2ppl.pdf

Next Week

- Maximum Likelihood