### BIOS 545: Statistical Analysis

#### Dane Van Domelen

Department of Biostatistics and Bioinformatics Rollins School of Public Health Emory University Atlanta, GA

April 9, 2018

# Statistical analysis in R

- Analyzing data is what (most) statisticians do.
- General procedure:
  - 1. Load data into R.
  - 2. Clean data (boring am I right??)
  - 3. Try to answer a research question.

#### Format of lecture

- Basic idea: Go through various research questions on a particular dataset to illustrate data analysis tools in R.
- Dataset: NHANES physical activity.
- General procedure for each research question:
  - (1) Visualize data (review!)
  - (2) Estimate parameter of interest
  - (3) Perform hypothesis test

### NHANES dataset

- <u>National Health And Nutrition Examination Survey</u>
  - Cross-sectional study in the US.
  - $n \approx 10,000$  in each 2-year cycle.
  - Demographics, questionnaires, lab tests, etc.
  - Publicly available! https://www.cdc.gov/nchs/nhanes/

# Putting dataset together (FYI)

```
# Install/load packages
install github("vandomed/nhanesaccel")
install_github("vandomed/nhanesdata")
library("nhanesaccel")
library("nhanesdata")
# Process NHANES 2003-2006 data
nhanes.pa <- process nhanes(waves = 1, valid wk days = 5, valid we days = 2,
                            weekday weekend = TRUE, brevity = 2)
# Subset participants with usable data, and only keep certain variables
nhanes.pa <- nhanes.pa %>%
 filter(include == 1) %>%
 select(seqn, cpm, sed_min, guideline_min, wk_cpm, we_cpm)
# Merge in demographics and body measurements datasets
data(demo_c)
data(bmx c)
names(demo c) <- tolower(names(demo c))
names(bmx_c) <- tolower(names(bmx_c))</pre>
nhanes <- nhanes.pa %>% inner_join(demo_c) %>% inner_join(bmx_c)
# Subset variables of interest and create some factors
nhanes <- nhanes %>%
 select(segn, riagendr, ridagevr, ridreth2, dmdeduc2, indfmpir, bmxbmi,
         bmxwaist, cpm, sed_min, guideline_min, wk_cpm, we_cpm) %>%
 rename (sex = riagendr, age = ridageyr, eth = ridreth2, educ = dmdeduc2,
         pir = indfmpir, bmi = bmxbmi, waist = bmxwaist) %>%
 mutate(sex = factor(sex, levels = c(1, 2), labels = c("Male", "Female")))
```

#### Initial look at dataset

[1] 7176 13

# Download dataset from website

```
load(url(
   "https://github.com/vandomed/vandomed.github.io/raw/master/nhanes.rda"))
# Look at data structure
dim(nhanes)
```

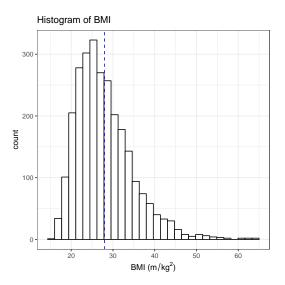
```
head(nhanes, 3)

seqn sex age eth educ pir bmi waist cpm sed_min guideline_min
1 21005 Male 19 2 NA 2.44 50.85 135.9 609.54 458.67 19.333
2 21006 Female 16 2 NA 2.47 20.78 73.6 145.46 591.50 2.750
3 21007 Female 14 1 NA 1.60 18.43 69.5 402.28 482.71 0.000
wk_cpm we_cpm
1 649.29 530.04
2 171.18 119.74
3 366.36 492.09
```

**Research question:** What is the mean BMI of American adults age 18-50?

#### Visualization

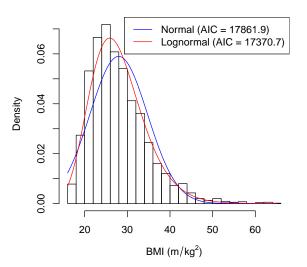
### Visualization



### Normal or lognormal?

# Normal or lognormal?

#### Histogram of BMI



- Statistical setup:
  - Let X = BMI.
  - Assume  $X_1,...,X_n \stackrel{\textit{iid}}{\sim} (\mu,\sigma^2)$
- Estimators:

$$\hat{\mu} = \bar{X}$$

95% CI for 
$$\mu : \bar{X} \pm \frac{t_{(.975,n-1)}s}{\sqrt{n}}$$

```
# Calculate sample mean
(x.bar <- mean(nhanes.adults$bmi, na.rm = T))
[1] 27.979</pre>
```

```
# Calculate 95% CI manually
s <- sd(nhanes.adults$bmi, na.rm = T)
n <- sum(! is.na(nhanes.adults$bmi))
t <- qt(p = 0.975, df = n - 1)
c(x.bar - t * s / sqrt(n), x.bar + t * s / sqrt(n))</pre>
[1] 27.723 28.236
```

```
# Better to use built-in R function!
t.test(nhanes.adults$bmi)
One Sample t-test
data: nhanes.adults$bmi
t = 214, df = 2680, p-value <2e-16
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
 27.723 28.236
sample estimates:
mean of x
   27.979
```

# Hypothesis test

Suppose we want to test:

$$H_0$$
 :  $\mu = 25$ 

$$H_A$$
 :  $\mu \neq 25$ 

# Hypothesis testing

```
(ttest.fit <- t.test(nhanes.adults$bmi, mu = 25))

One Sample t-test

data: nhanes.adults$bmi
t = 22.8, df = 2680, p-value <2e-16
alternative hypothesis: true mean is not equal to 25
95 percent confidence interval:
27.723 28.236
sample estimates:
mean of x
27.979</pre>
```

# Hypothesis testing

```
names(ttest.fit)
[1] "statistic" "parameter" "p.value"
                                             "conf.int" "estimate"
[6] "null.value" "alternative" "method"
                                             "data.name"
ttest.fit$estimate
mean of x
  27.979
ttest.fit$conf.int
[1] 27.723 28.236
attr(,"conf.level")
[1] 0.95
```

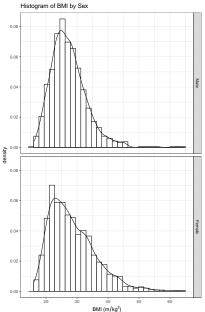
```
ttest.fit$p.value
```

[1] 2.3446e-105

**Research question:** In American adults age 18-50, is the population mean BMI for males the same as for females?

#### Visualization

### Visualization



- Statistical setup:
  - Let X = BMI for males, Y = BMI for females.
  - $X_1, ..., X_{n_m} \stackrel{iid}{\sim} (\mu_m, \sigma_m^2)$
  - $Y_1, ..., Y_{n_f} \stackrel{iid}{\sim} (\mu_f, \sigma_f^2)$
- Parameters/Estimators:
  - $\mu_{\Delta} = \mu_{m} \mu_{f}$
  - $\widehat{\mu}_{\Delta} = \overline{X} \overline{Y}$
  - 95% CI based on t-distribution  $\Rightarrow$  2 versions

```
# Fit two-sample t-test by giving t.test two vectors
(ttest.fit <- t.test(nhanes.adults$bmi[nhanes.adults$sex == "Male"],</pre>
                     nhanes.adults$bmi[nhanes.adults$sex == "Female"]))
Welch Two Sample t-test
data: nhanes.adults$bmi[nhanes.adults$sex == "Male"] and nhanes.adults$bmi[nha
t = -4.65, df = 2630, p-value = 3.6e-06
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-1.70153 - 0.69144
sample estimates:
mean of x mean of y
  27.351 28.548
```

```
# Fit two-sample t-test using formula notation (easier!)
(ttest.fit <- t.test(bmi ~ sex, data = nhanes.adults))</pre>
Welch Two Sample t-test
data: bmi by sex
t = -4.65, df = 2630, p-value = 3.6e-06
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-1.70153 - 0.69144
sample estimates:
  mean in group Male mean in group Female
              27.351
                                    28.548
```

# Hypothesis testing

Already saw results for two-sample t-test:

$$H_0: \mu_{\Delta} = 0$$

$$H_A: \mu_{\Delta} \neq 0$$

 $\Rightarrow$  Can also test whether  $\mu_{\Delta}$  equals some non-zero value, but this is less common.

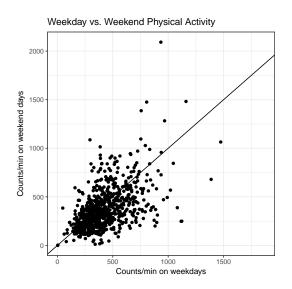
### In-class activity

- (1) Find out whether we assumed equal variance.
- (2) Decide whether we should assume equal variance.
- (3) Test  $H_0: \mu_{\Delta} = -1$ , using appropriate test.

**Research question:** Are American adolescents age 13-17 more physically active on weekdays, or on weekend days?

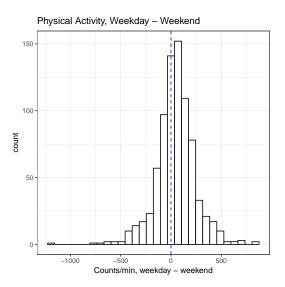
## Visualization #1: Scatterplot

### Visualization #1: Scatterplot



## Visualization #2: Histogram

## Visualization #2: Histogram



- Statistical setup:
  - Let X = Difference between average weekday PA and average weekend PA.

• 
$$X_1,...,X_n \stackrel{iid}{\sim} (\mu,\sigma^2)$$

• Estimators:

$$\hat{\mu} = \bar{X}$$

95% CI for 
$$\mu: \bar{X} \pm \frac{t_{(.975,n-1)}s}{\sqrt{n}}$$

```
# Fit paired t-test by giving t.test two vectors
t.test(nhanes.adol$we_cpm, nhanes.adol$wk_cpm, paired = T)

Paired t-test

data: nhanes.adol$we_cpm and nhanes.adol$wk_cpm
t = -6.7, df = 796, p-value = 3.9e-11
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
    -60.469 -33.072
sample estimates:
mean of the differences
    -46.77
```

```
# Fit paired t-test by giving t.test single vector of differences
t.test(nhanes.adol$cpm_diff)
One Sample t-test
data: nhanes.adol$cpm_diff
t = 6.7, df = 796, p-value = 3.9e-11
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
33.072 60.469
sample estimates:
mean of x
   46.77
```

# Hypothesis testing

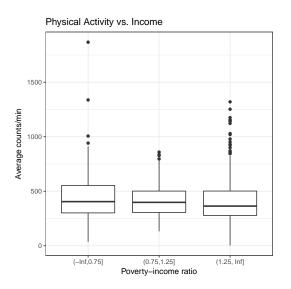
Already saw results for paired t-test:

$$H_0$$
 :  $\mu = 0$ 

$$H_A$$
 :  $\mu \neq 0$ 

Research question: Does physical activity differ by family income level (low, medium, high) in American adolescents?

#### Visualization



### Parameter estimation

- Statistical setup:
  - Let  $X_{i,j}$  = Average physical activity for  $j^{th}$  participant in  $i^{th}$  PIR group, i = 1, 2, 3;  $j = 1, ..., n_i$
  - Assume  $X_{i,j} \stackrel{ind}{\sim} (\mu_i, \sigma^2)$ , i = 1, 2, 3
- Estimators:
  - $\hat{\mu}_i = \bar{X}_i$
  - 95% CI for each  $\mu_i: \bar{X}_i \pm \frac{t_{(.975,n_i-1)}s_i}{\sqrt{n_i}}$

### Parameter estimation

#### Parameter estimation

```
# Interval estimates for mu's
tapply(nhanes.adol$cpm, nhanes.adol$pir.f,
       function(x) t.test(x)$conf.int)
$`(-Inf,0.75]`
[1] 411.54 465.12
attr(,"conf.level")
[1] 0.95
$`(0.75,1.25]`
[1] 390.09 438.85
attr(,"conf.level")
[1] 0.95
$`(1.25, Inf]`
[1] 394.84 424.05
attr(,"conf.level")
[1] 0.95
```

Natural thing to test:

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

 $H_A$ : Not all  $\mu's$  equal

 $\Rightarrow$  One-way ANOVA

```
# Fit ANOVA
anova.fit <- aov(cpm ~ pir.f, data = nhanes.adol)
summary(anova.fit)

Df Sum Sq Mean Sq F value Pr(>F)
pir.f 2 143785 71892 1.99 0.14
Residuals 1057 38259088 36196
231 observations deleted due to missingness
```

```
# Multiple comparisons
TukeyHSD(anova.fit)

Tukey multiple comparisons of means
95% family-wise confidence level

Fit: aov(formula = cpm ~ pir.f, data = nhanes.adol)

$pir.f

diff lwr upr p adj
(0.75,1.25]-(-Inf,0.75] -23.8621 -69.217 21.4927 0.43287
(1.25, Inf]-(-Inf,0.75] -28.8918 -63.014 5.2303 0.11582
(1.25, Inf]-(0.75,1.25] -5.0297 -43.696 33.6368 0.94992
```

**Research question:** In older American males, Is there an association between race/ethnicity (4 levels) and obesity status (3 levels)?

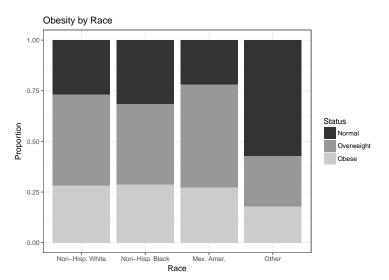
### Generate variables

```
# Contingency table with frequencies
(table.freq <- table(nhanes.om$race.f, nhanes.om$obesity.f))

Normal Overweight Obese
Non-Hisp. White 124 209 131
Non-Hisp. Black 34 43 31
Mex. Amer. 36 83 45
Other 16 7 5
```

```
# Contingency table with row proportions
(table.rowprops <- prop.table(table.freq, margin = 1))

Normal Overweight Obese
Non-Hisp. White 0.26724  0.45043 0.28233
Non-Hisp. Black 0.31481  0.39815 0.28704
Mex. Amer.  0.21951  0.50610 0.27439
Other  0.57143  0.25000 0.17857
```



Typical test for two categorical variables:

 $H_0$ : Race and obesity are not associated.

 $H_A$ : Race and obesity are associated.

⇒ Chi-square test of association

```
# Chi-square test of association
chisq.test(nhanes.om$race.f, nhanes.om$obesity.f)

Pearson's Chi-squared test

data: nhanes.om$race.f and nhanes.om$obesity.f
X-squared = 16.9, df = 6, p-value = 0.0098
```

```
# Chi-square test of association
chisq.test(nhanes.om$race.f, nhanes.om$obesity.f)

Pearson's Chi-squared test

data: nhanes.om$race.f and nhanes.om$obesity.f
X-squared = 16.9, df = 6, p-value = 0.0098
```

- $\Rightarrow$  P-value suggests race is significantly assoc. with obesity.
- ⇒ Chi-square test valid?

```
# Look at expected cell counts for each cell

(expected.counts <- matrix(rowSums(table.freq), ncol = 1) %*%

colSums(table.freq) / sum(table.freq))

[,1] [,2] [,3]

[1,] 127.5393 207.707 128.7539

[2,] 29.6859 48.346 29.9686

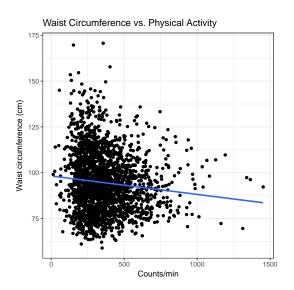
[3,] 45.0785 73.414 45.5079

[4,] 7.6963 12.534 7.7696
```

## In-class activity

- (1) Figure out what small-sample test we could use here.
- (2) Figure out what R function does it.
- (3) Perform test  $\Rightarrow$  same conclusion as Chi-square?

**Research question:** In American adults age 18-50, is there a correlation between physical activity and waist circumference?



# Correlation analysis

- Statistical setup:
  - Let X = physical activity, Y = waist circ.
  - Assume  $(X_1, Y_1), ..., (X_n, Y_n) \stackrel{iid}{\sim} (\mu, \Sigma)$ .
- Parameter of interest:

$$\rho_{xy} = \mathsf{Cor}(X, Y) = \frac{\mathsf{Cov}(X, Y)}{\sqrt{V(X)V(Y)}} = \frac{E(XY) - E(X)E(Y)}{\sqrt{V(X)V(Y)}}$$

Hypothesis test:

$$H_0: \rho = 0$$

$$H_A: \rho \neq 0$$

## Correlation analysis

## Correlation analysis

```
# Calculate Spearman correlation coefficient
cor.test(nhanes.adults$cpm, nhanes.adults$waist, method = "spearman")

Spearman's rank correlation rho

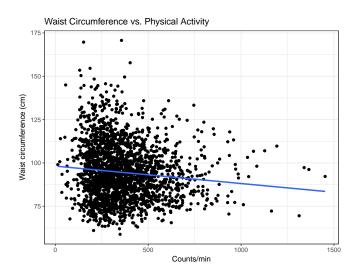
data: nhanes.adults$cpm and nhanes.adults$waist
S = 2.16e+09, p-value = 9.1e-07
alternative hypothesis: true rho is not equal to 0
sample estimates:
    rho
-0.10275
```

- Statistical setup:
  - Assume  $Y_i = \beta_0 + \beta_1 X_i + \epsilon_i, \ \epsilon_i \stackrel{iid}{\sim} (0, \sigma^2)$
- Parameters/estimation:
  - $\beta_0=$  intercept,  $\beta_1=$  slope
  - OLS:  $\hat{\beta} = (X^T X)^{-1} X^T y$
- Hypothesis test of primary interest:

$$H_0: \beta_1 = 0$$

$$H_A: \beta_1 \neq 0$$

# Intercept and slope



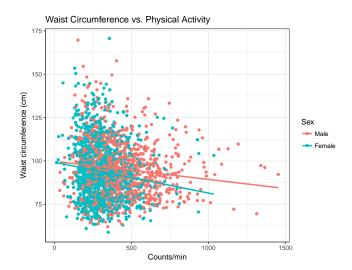
```
# Fit linear regression for waist circumference vs. physical activity
linear.fit <- lm(waist ~ cpm, data = nhanes.adults)
summary(linear.fit)</pre>
```

```
Call:
lm(formula = waist ~ cpm, data = nhanes.adults)
Residuals:
   Min 10 Median 30 Max
-35.86 -11.74 -1.65 9.72 76.12
Coefficients:
           Estimate Std. Error t value Pr(>|t|)
(Intercept) 98.16700 0.79261 123.85 < 2e-16 ***
           -0.01006 0.00199 -5.06 4.6e-07 ***
cpm
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 15.9 on 2271 degrees of freedom
  (440 observations deleted due to missingness)
Multiple R-squared: 0.0111, Adjusted R-squared: 0.0107
F-statistic: 25.6 on 1 and 2271 DF, p-value: 4.57e-07
```

```
# Divide CPM by 100 to make slope easier to interpret
nhanes.adults$cpm_100 <- nhanes.adults$cpm / 100
linear.fit <- lm(waist ~ cpm_100, data = nhanes.adults)
summary(linear.fit)</pre>
```

```
Call:
lm(formula = waist ~ cpm_100, data = nhanes.adults)
Residuals:
   Min 10 Median 30 Max
-35.86 -11.74 -1.65 9.72 76.12
Coefficients:
           Estimate Std. Error t value Pr(>|t|)
(Intercept) 98.167 0.793 123.85 < 2e-16 ***
cpm_100 -1.006 0.199 -5.06 4.6e-07 ***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 15.9 on 2271 degrees of freedom
  (440 observations deleted due to missingness)
Multiple R-squared: 0.0111, Adjusted R-squared: 0.0107
F-statistic: 25.6 on 1 and 2271 DF, p-value: 4.57e-07
```

**Research question:** Does the relationship between physical activity and waist circumference differ by sex?



- Statistical setup:
  - Let X = physical activity, Y = waist circumference, and M = 1 if male, 0 if female.
  - Assume  $Y_i = \beta_0 + \beta_1 X_i + \beta_2 M_i + \beta_3 X_i M_i + \epsilon_i, \ \epsilon_i \stackrel{iid}{\sim} (0, \sigma^2)$
- Hypothesis test of primary interest:

$$H_0: \beta_3 = 0$$

$$H_A: \beta_3 \neq 0$$

• What does it mean if  $\beta_3 = 0$ ?

```
# Fit model with interaction term
nhanes.adults$male <- ifelse(nhanes.adults$sex == "Male", 1, 0)
linear.fit <- lm(waist ~ cpm + male + cpm * male, data = nhanes.adults)
summary(linear.fit)</pre>
```

```
Call:
lm(formula = waist ~ cpm + male + cpm * male, data = nhanes.adults)
Residuals:
  Min 10 Median 30 Max
-34.59 -11.55 -1.77 9.45 77.79
Coefficients:
          Estimate Std. Error t value Pr(>|t|)
cpm -0.01782 0.00364 -4.90 1e-06 ***
male 0.56108 1.68716 0.33 0.739
cpm:male 0.00732 0.00443 1.65 0.099.
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 15.8 on 2269 degrees of freedom
 (440 observations deleted due to missingness)
Multiple R-squared: 0.0208, Adjusted R-squared: 0.0195
F-statistic: 16.1 on 3 and 2269 DF, p-value: 2.4e-10
```

**Question:** What if we drop the interaction term? It was not significant after all.

• Previously, with interaction term:

• 
$$Y_i = \beta_0 + \beta_1 X_i + \beta_2 M_i + \beta_3 X_i M_i + \epsilon_i$$
,  $\epsilon_i \stackrel{\text{iid}}{\sim} (0, \sigma^2)$ 

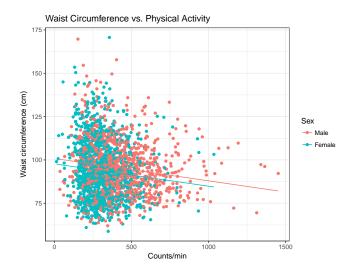
Now:

• 
$$Y_i = \beta_0^* + \beta_1^* X_i + \beta_2^* M_i + \epsilon_i$$
,  $\epsilon_i \stackrel{iid}{\sim} (0, \sigma^{*2})$ 

- Parameters:
  - Now, what is the slope for males? Females?
  - Are the regression lines the same?

```
# Scatterplot with regression line that does not include interaction
fit <- lm(waist ~ cpm + as.factor(male), data = nhanes.adults)
nhanes.adults$pred_waist <- predict(fit, newdata = nhanes.adults)

ggplot(nhanes.adults, aes(cpm, waist, color = sex)) +
    geom_point() +
    labs(title = "Waist Circumference vs. Physical Activity",
        x = "Counts/min",
        y = "Waist circumference (cm)",
        color = "Sex") +
    geom_line(aes(y = pred_waist)) +
    theme_bw()</pre>
```



```
# Fit model without interaction term
linear.fit <- lm(waist ~ cpm + male, data = nhanes.adults)
summary(linear.fit)</pre>
```

```
Call:
lm(formula = waist ~ cpm + male, data = nhanes.adults)
Residuals:
  Min 10 Median 30 Max
-34.43 -11.63 -1.82 9.47 77.57
Coefficients:
           Estimate Std. Error t value Pr(>|t|)
(Intercept) 97.72059 0.79575 122.80 < 2e-16 ***
       -0.01289 0.00208 -6.20 6.8e-10 ***
cpm
male
        3.09604 0.69763 4.44 9.5e-06 ***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 15.8 on 2270 degrees of freedom
 (440 observations deleted due to missingness)
Multiple R-squared: 0.0196, Adjusted R-squared: 0.0188
F-statistic: 22.7 on 2 and 2270 DF, p-value: 1.66e-10
```

# Final thoughts

- The internet exists. No need to memorize!
- Function help files are...helpful.
- In most cases, a graph is (at least) as good as a test.
- Make graph  $\Rightarrow$  eyeball association  $\Rightarrow$  perform test.

## Some other topics

- Survival analysis (survival package)
- Longitudinal analysis (nlme, lme4, gee packages)
- Complex survey analysis (survey package)

#### Lab

You may or may not be familiar with logistic regression. Logistic regression is what you use to test whether one or more variables are associated with a binary outcome variable.

For logistic regression with a binary outcome Y and two predictors  $X_1$  and  $X_2$ , we assume the following model:

$$\log[\frac{P(Y_i=1)}{1-P(Y_i=1)}] = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i}$$

In other words, we assume there is a linear relationship between each predictor and the log-odds of Y.

If  $\beta_1=0$ , then  $X_1$  is not associated with Y. If  $\beta_1$  is positive, then people with higher values for  $X_1$  are more likely to experience the outcome than people with lower values of  $X_1$ ; and vice versa if  $\beta_1$  is negative.

In this lab, you will learn how to use the glm function to fit a logistic regression model in R. It works like lm, but can handle various regression models, not just linear regression.

Follow these steps to test whether sex and waist circumference are associated with odds of meeting the US physical activity guidelines for adults ( $\geq$  150 minutes of exercise per week).

- 1. Run code from slide 6 to download nhanes data frame.
- 2. Subset data for participants age 18-65.
- 3. Create variable that is 1 if guideline\_min > 21.4, and 0 otherwise.
- 4. Look at help file for glm and see what input you have to specify to get R to do logistic regression.
- 5. Fit and interpret logistic regression for meeting guidelines vs. sex and waist circumference.
- 6. Re-fit model with sex-by-waist circumference interaction.

Does relationship between waist circumference and odds of meeting the guidelines differ by sex? If so, is the association stronger in males or females?