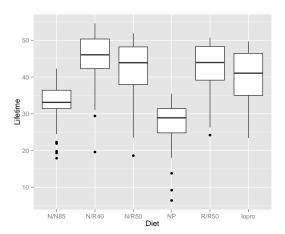
# STAT 401A - Statistical Methods for Research Workers One-way ANOVA

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### Mice lifetimes



# Simple hypothesis

Consider the one-way ANOVA model:  $Y_{ij} \sim N(\mu_j, \sigma^2)$  where  $j = 1, \dots, J$ .

Here are a few simple alternative hypotheses:

- Mean lifetimes for N/R50 and R/R50 diet are different.
- Mean lifetimes for N/R40 is different than for N/R50 and R/R50 combined.
- Mean lifetimes for high calorie (NP and N/N85) diets is different than for low calorie diets combined.

$$H_0: \gamma = 0$$
  $H_1: \gamma \neq 0:$  
$$\gamma_1 = \mu_{R/R50} - \mu_{N/R50}$$
 
$$\gamma_2 = \frac{1}{2}(\mu_{N/R50} + \mu_{R/R50}) - \mu_{N/R40}$$
 
$$\gamma_3 = \frac{1}{2}(\mu_{NP} + \mu_{N/N85}) - \frac{1}{4}(\mu_{N/R50} + \mu_{R/R50} + \mu_{N/R40} + \mu_{lopro})$$

### Contrasts

### Definition

A linear combination of group means has the form

$$\gamma = C_1 \mu_1 + C_2 \mu_2 + \ldots + C_J \mu_J$$

where  $\mathcal{C}_j$  are known coefficients and  $\mu_j$  are the unknown population means.

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### Definition

A linear combination with  $C_1 + C_2 + \cdots + C_J = 0$  is a contrast.

**Remark** Contrast interpretation is usually best if  $|C_1| + |C_2| + \cdots + |C_J| = 2$ , i.e. both the positive and negative coefficients sum to 1.

### Inference on contrasts

$$\gamma = C_1 \mu_1 + C_2 \mu_2 + \dots + C_J \mu_J$$

Estimated by

$$g = C_1 \overline{Y}_1 + C_2 \overline{Y}_2 + \dots + C_J \overline{Y}_J$$

with standard error

$$SE(g) = s_p \sqrt{\frac{C_1^2}{n_1} + \frac{C_2^2}{n_2} + \dots + \frac{C_J^2}{n_J}}$$

t-statistic (compare to  $t_{n-J}$ ) and CI:

$$t = \frac{g}{SE(g)}$$
  $g \pm t_{n-J}(1 - \alpha/2)SE(g)$ 

### Contrasts for mice lifetime dataset

### For these contrasts:

- Mean lifetimes for N/R50 and R/R50 diet are different.
- Mean lifetimes for N/R40 is different than for N/R50 and R/R50 combined.
- Mean lifetimes for high calorie (NP and N/N85) diets is different than for low calorie diets combined.

$$H_0: \gamma = 0$$
  $H_1: \gamma \neq 0:$ 

$$\begin{array}{ll} \gamma_1 &= \mu_{R/R50} - \mu_{N/R50} \\ \gamma_2 &= \frac{1}{2} (\mu_{N/R50} + \mu_{R/R50}) - \mu_{N/R40} \\ \gamma_3 &= \frac{1}{2} (\mu_{NP} + \mu_{N/N85}) - \frac{1}{4} (\mu_{N/R50} + \mu_{R/R50} + \mu_{N/R40} + \mu_{lopro}) \end{array}$$

	N/N85	N/R40	N/R50	NP	R/R50	lopro
early rest - none @ 50kcal	0.00	0.00	-1.00	0.00	1.00	0.00
40kcal/week - 50kcal/week	0.00	1.00	-0.50	0.00	-0.50	0.00
lo cal - hi cal	-0.50	0.25	0.25	-0.50	0.25	0.25

# Mice liftime examples

	Diet	n	mean	sd
1	N/N85	57	32.69	5.13
2	N/R40	60	45.12	6.70
3	N/R50	71	42.30	7.77
4	NP	49	27.40	6.13
5	R/R50	56	42.89	6.68
6	lopro	56	39.69	6.99

### Contrasts:

	g	SE(g)	t	р	L	U
early rest - none @ 50kcal	0.59	1.19	0.49	0.62	-1.76	2.94
40kcal/week - 50kcal/week	2.53	1.05	2.41	0.02	0.46	4.59
lo cal - hi cal	12.45	0.78	15.96	0.00	10.92	13.98

SAS

```
DATA case0501;
  INFILE 'case0501.csv' DSD FIRSTOBS=2;
  INPUT lifetime diet $;
PROC MEANS DATA=case0501;
  CLASS diet;
  VAR lifetime;
  RUN;
```

The MEANS Procedure Analysis Variable : lifetime

	N					
diet	0bs	N	Mean	Std Dev	Minimum	Maximum
N/N85	57	 57	32.6912281	5.1252972	17.9000000	42.3000000
N/R40	60	60	45.1166667	6.7034058	19.6000000	54.6000000
N/R50	71	71	42.2971831	7.7681947	18.6000000	51.9000000
NP	49	49	27.4020408	6.1337010	6.4000000	35.5000000
R/R50	56	56	42.8857143	6.6831519	24.2000000	50.7000000
lopro	56	56	39.6857143	6.9916945	23.4000000	49.7000000

SAS

```
PROC GLM;
 CLASS diet:
 MODEL lifetime = diet / CLPARM;
 ESTIMATE 'early rest - none @ 50kcal' diet 0 1 -1 0 0 0;
 ESTIMATE '40kcal/week - 50kcal/week' diet 0 2 -1 0 -1 0 / DIVISOR = 2:
 ESTIMATE 'lo cal - hi cal' diet -2 1 1 -2 1 1 / DIVISOR = 4;
 RUN;
 QUIT;
```

#### The GLM Procedure

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	12733.94181	2546.78836	57.10	<.0001
Error	343	15297.41532	44.59888		
Corrected Total	348	28031.35713			

		Standard		
Parameter	Estimate	Error	t Value	Pr >  t
early rest - none @ 50kcal	0.5885312	1.19355007	0.49	0.6223
40kcal/week - 50kcal/week	2.5252180	1.04854904	2.41	0.0166
lo cal - hi cal	12.4496851	0.78001425	15.96	<.0001

Parameter	95% Confider	nce Limits
early rest - none @ 50kcal	-1.7590676	2.9361299
40kcal/week - 50kcal/week	0.4628224	4.5876136
lo cal - hi cal	10.9154718	13.9838985

C+----

R

```
library(multcomp)
m = lm(Lifetime~Diet-1, case0501) # The -1 indicates no intercept (see Ch 7)
summary(m)
Call:
lm(formula = Lifetime ~ Diet - 1, data = case0501)
Residuals:
       10 Median 30
   Min
                                Max
-25.517 -3.386 0.814 5.183 10.014
Coefficients:
         Estimate Std. Error t value Pr(>|t|)
DietN/N85 32.691
                     0.885
                             37.0
                                  <2e-16 ***
DietN/R40 45.117
                     0.862 52.3
                                  <2e-16 ***
DietN/R50 42.297 0.793 53.4
                                  <2e-16 ***
DietNP
      27.402 0.954
                             28.7
                                  <2e-16 ***
DietR/R50 42.886 0.892
                             48.1
                                  <2e-16 ***
                     0.892 44.5 <2e-16 ***
Dietlopro 39.686
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 6.68 on 343 degrees of freedom
Multiple R-squared: 0.972, Adjusted R-squared: 0.972
F-statistic: 2.01e+03 on 6 and 343 DF, p-value: <2e-16
K
```

```
N/N85 N/R40 N/R50 NP R/R50 lopro
early rest - none @ 50kcal 0.0 0.00 -1.00 0.0 1.00 0.00
40kcal/week - 50kcal/week 0.0 1.00 -0.50 0.0 -0.50 0.00
                         -0.5 0.25 0.25 -0.5 0.25 0.25
lo cal - hi cal
```

```
t = glht(m, linfct=K)
summarv(t)
Simultaneous Tests for General Linear Hypotheses
Fit: lm(formula = Lifetime ~ Diet - 1, data = case0501)
Linear Hypotheses:
                             Estimate Std. Error t value Pr(>|t|)
early rest - none @ 50kcal == 0 0.589 1.194 0.49 0.946
40kcal/week - 50kcal/week == 0 2.525 1.049 2.41 0.049 *
                      12.450 0.780 15.96 <1e-04 ***
lo cal - hi cal == 0
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)
confint(t, calpha=univariate calpha())
Simultaneous Confidence Intervals
Fit: lm(formula = Lifetime ~ Diet - 1, data = case0501)
Quantile = 1.967
95% confidence level
Linear Hypotheses:
                             Estimate lwr upr
```

early rest - none @ 50kcal == 0 0.589 -1.759 2.936 40kcal/week - 50kcal/week == 0 2.525 0.463 4.588 lo cal - hi cal == 0 12.450 10.915 13.984

### Summary

- Contrasts are linear combinations that sum to zero
- t-test tools are used to calculate pvalues and confidence intervals

# SAS code and output for one-way ANOVA

```
DATA mice;

INFILE 'case0501.csv' DSD FIRSTOBS=2;

INPUT lifetime diet $;

PROC GLM DATA=mice;

CLASS diet;

MODEL lifetime = diet;

LSMEANS diet / ADJUST=T;

RUN:
```

#### The GLM Procedure

#### Dependent Variable: lifetime

		Sum oi			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	12733.94181	2546.78836	57.10	<.0001
Error	343	15297.41532	44.59888		
Corrected Total	348	28031 35713			

# SAS code and output for pairwise comparisons

The GLM Procedure Least Squares Means

diet	lifetime LSMEAN	LSMEAN Number
N/N85	32.6912281	1
N/R40	45.1166667	2
N/R50	42.2971831	3
NP	27.4020408	4
R/R50	42.8857143	5
lopro	39.6857143	6

Least Squares Means for effect diet Pr > |t| for HO: LSMean(i)=LSMean(j)

#### Dependent Variable: lifetime

i/j	1	2	3	4	5	6
1		<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001		0.0166	<.0001	0.0731	<.0001
3	<.0001	0.0166		<.0001	0.6223	0.0293
4	<.0001	<.0001	<.0001		<.0001	<.0001
5	<.0001	0.0731	0.6223	<.0001		0.0117
6	<.0001	<.0001	0.0293	<.0001	0.0117	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

### Statistical testing errors

#### Definition

A type I error occurs when a true null hypothesis is rejected.

### Definition

A type II error occurs when a false null hypothesis is not rejected. Power is one minus the type II error probability.

**Remark** We set  $\alpha$  to control the type I error probability. If we set  $\alpha=0.05$ , then we will incorrectly reject a true null hypothesis 5% of the time.

### Definition

The familywise error rate is the probability of rejecting at least one true null hypothesis.

# Type I error for all pairwise comparisons of J groups

How many combinations when choosing 2 items out of J?

$$\binom{J}{2} = \frac{J!}{2!(J-2)!}.$$

If J=6, then there are 15 different comparison of means. If we set  $\alpha=0.05$  as our significance level, then individually each test will only incorrectly reject 5% of the time.

If we have 15 tests and use  $\alpha = 0.05$ , what is the familywise error rate?

$$1 - (1 - 0.05)^{15} = 1 - (0.95)^{15} = 1 - 0.46 = 0.54$$

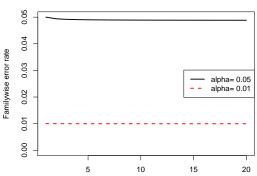
So there is a greater than 50% probability of falsely rejecting a true null hypothesis!

### Bonferroni correction

#### Definition

If we do m tests and want the familywise error rate to be  $\alpha$ , the Bonferroni correction uses  $\alpha/m$  for each individual test. The familywise error rate, for independent tests, is  $1 - (1 - \alpha/, m)^m$ .

#### Bonferroni familywise error rate



# SAS code and output for pairwise comparisons

### Compare the unadjusted pvalues to $\alpha/15 = 0.05/15 = 0.0033$ .

Least Squares Means for effect diet Pr > |t| for HO: LSMean(i)=LSMean(j)

#### Dependent Variable: lifetime

i/j	1	2	3	4	5	6
1		<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001		0.0166	<.0001	0.0731	<.0001
3	<.0001	0.0166		<.0001	0.6223	0.0293
4	<.0001	<.0001	<.0001		<.0001	<.0001
5	<.0001	0.0731	0.6223	<.0001		0.0117
6	<.0001	<.0001	0.0293	<.0001	0.0117	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

Now 2-3, 3-6, and 5-6 are no longer significant.

# SAS code and output for one-way ANOVA

If you use SAS to do the adjustment, compare pvalues to  $\alpha = 0.05$ .

```
DATA mice;
  INFILE 'case0501.csv' DSD FIRSTOBS=2:
  INPUT lifetime diet $:
PROC GLM DATA=mice:
  CLASS diet:
  MODEL lifetime = diet;
  LSMEANS diet / ADJUST=BON;
  RUN:
                                Least Squares Means for effect diet
                                Pr > |t| for HO: LSMean(i)=LSMean(i)
                                    Dependent Variable: lifetime
    i/j
                      1
                                                    3
                                < .0001
                                               < .0001
                                                              0.0009
                                                                             < .0001
                                                                                            < .0001
       2
                 <.0001
                                               0.2488
                                                              <.0001
                                                                             1.0000
                                                                                            0.0002
       3
                 < .0001
                                0.2488
                                                              <.0001
                                                                             1.0000
                                                                                            0.4402
                 0.0009
                                < .0001
                                               < .0001
                                                                             < .0001
                                                                                            <.0001
                 < .0001
                                1.0000
                                               1.0000
                                                              < .0001
                                                                                            0.1751
                 < .0001
                                0.0002
                                                              < .0001
                                                                             0.1751
                                               0.4402
```

### Comments on the Bonferroni correction

**Remark** The Bonferroni correction can be used in any situation. In particular, it can be used on unadjusted pvalues reported in an article that has many tests by comparing their pvalues to  $\alpha/m$  where m is the number of tests they perform.

**Remark** The Bonferroni correction is (in general) the most conservative multiple comparison adjustment we will discuss, i.e. it will lead to the least null hypothesis rejections.

# Constructing multiple confidence intervals

Confidence interval for the difference between group i and group i':

$$\overline{Y}_i - \overline{Y}_{i'} \pm M s_p \sqrt{\frac{1}{n_i} + \frac{1}{n_{i'}}}$$

where M is a multiplier that depends on the adjustment procedure:

Procedure	M	Use
LSD	$t_{n-J}(1-\alpha)$	After significant <i>F</i> -test
		(no adjustment)
Dunnett		Compare all groups to control
Tukey-Kramer	$q_{J,n-J}(1-lpha)/\sqrt{2}$	All pairwise comparisons
Scheffé	$\sqrt{(J-1)F_{(J-1,n-J)}(1-\alpha)}$	All contrasts
Bonferroni	$t_{n-J}(1-\alpha/2k)$	k tests
	k = J(J-1)/2	(most generic)

# SAS code and output for one-way ANOVA

```
DATA mice;
INFILE 'case0501.csv' DSD FIRSTOBS=2;
INPUT lifetime diet $;

PROC GLM DATA=mice;
CLASS diet;
MODEL lifetime = diet;
LSMEANS diet / CL ADJUST=TUKEY;
RUN;

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer
```

#### Dependent Variable: lifetime

i/j	1	2	3	4	5	$\epsilon$
1		<.0001	<.0001	0.0008	<.0001	<.0001
2	<.0001		0.1565	<.0001	0.4684	0.0002
3	<.0001	0.1565		<.0001	0.9964	0.2460
4	0.0008	<.0001	<.0001		<.0001	<.0001
5	<.0001	0.4684	0.9964	<.0001		0.1168
6	<.0001	0.0002	0.2460	<.0001	0.1168	

# SAS code and output for one-way ANOVA

#### The GLM Procedure Least Squares Means

Adjustment for Multiple Comparisons: Tukey-Kramer

#### Least Squares Means for Effect diet

		Difference	Simultaneous 95%			
		Between	Confidence	Limits for		
i	j	Means	LSMean(i)-	-LSMean(j)		
1	2	-12.425439	-15.965442	-8.885435		
1	3	-9.605955	-13.009741	-6.202169		
1	4	5.289187	1.560626	9.017749		
1	5	-10.194486	-13.795557	-6.593416		
1	6	-6.994486	-10.595557	-3.393416		
2	3	2.819484	-0.536769	6.175736		
2	4	17.714626	14.029406	21.399846		
2	5	2.230952	-1.325223	5.787128		
2	6	5.430952	1.874777	8.987128		
3	4	14.895142	11.340571	18.449714		
3	5	-0.588531	-4.009133	2.832070		
3	6	2.611469	-0.809133	6.032070		
4	5	-15.483673	-19.227592	-11.739755		
4	6	-12.283673	-16.027592	-8.539755		
5	6	3.200000	-0.416969	6.816969		

### How to incorporate multiple comparison adjustments

- Determine what tests are going to be run (before looking at the data)
- Oetermine which multiple comparison adjustment is the most relevant
- Use that adjustment and interpret your results