Topic: Designed experiments with multiple factors

- 2×2 experiments
- $a \times b$ experiments
- three-factor ANOVA
- nested vs. crossed designs (not described in packet)

An example of a 2×2 study

Cholesterol measurements for random samples of $n_j \equiv 7$ people from four populations given in the table below. Groups (cohorts) defined as follows:

I The population of women younger than 50
II The population of men younger than 50
III The population of women 50 years or older
IV The population of men 50 years or older

Sources of variability? ____________, ____________, ____________

Group	Cholesterol level							avg	std. dev.
I	221	213	202	183	185	197	162	$\bar{y}_I = 194.7$	$s = 20$
II	271	192	189	209	227	236	142	$\bar{y}_{II} = 209.4$	$s = 41$
III	262	193	224	201	161	178	265	$\bar{y}_{III} = 212.0$	$s = 40$
IV	192	253	248	278	232	267	289	$\bar{y}_{IV} = 251.3$	$s = 32$

One-way ANOVA Model:

$$\boxed{\begin{aligned} Y_{ij} &= \mu_i + E_{ij} && \text{each estimable} \\ &= \mu + \tau_i + E_{ij} && \text{each nonestimable} \end{aligned}}$$

$i = 1, 2, 3, 4 \quad j = 1, 2, \ldots, 7$ and E_{ij} i.i.d. $N(0, \sigma^2)$

Parameters: $\mu, \tau_1, \tau_2, \tau_3, \tau_4, \sigma^2$

One-way ANOVA table:

```
                              The GLM Procedure

                  Class          Levels     Values
                  cohort              4     I II III IV

                                     Sum of
 Source                    DF       Squares    Mean Square   F Value   Pr > F
 Model                      3   12280.85714     4093.61905      3.46   0.0323
 Error                     24   28434.57143     1184.77381
 Corrected Total           27   40715.42857

           R-Square     Coeff Var      Root MSE        y Mean
           0.301627      15.87245      34.42054      216.8571

 Source                    DF     Type I SS    Mean Square   F Value   Pr > F
 cohort                     3   12280.85714     4093.61905      3.46   0.0323
```

Some terminology

Definition: A ________________ in an experiment or study is a variable whose effect on the response is of primary interest. The values that a factor takes in the experiment are called factor ________________ or treatments.

Definition: In ________________________, experimental units are randomly assigned to factor levels, or treatment groups.

Note: The cholesterol study is NOT a completely randomized design, as randomization of subjects to different levels of AGE and GENDER isn't possible.

Definition: When the same number of units are used for each treatment, the design is ________________.

In one-way ANOVA of cholesterol data, COHORT is the factor, but it can be broken down into two factors in two-way ANOVA: AGE (factor A) and GENDER (factor B).

Definition: If there are observations at all combinations of all factors, the design is ________________, otherwise it is ________________.

1. Estimate the mean difference in cholesterol between young men and young women.
2. Estimate the mean difference between old men and old women.
3. Estimate the mean difference between men and women.
4. Estimate the mean difference between older and younger folks.
5. Estimate the mean difference between the differences estimated in 1. and 2.
6. Provide standard errors for all of these estimated contrasts
7. Specify the vectors defining these contrasts. For example, the first contrast of cohort means can be written

$$\theta_1 = (-1, 1, 0, 0)' \begin{pmatrix} \mu_1 \\ \mu_2 \\ \mu_3 \\ \mu_4 \end{pmatrix} = \mu_2 - \mu_1$$

Consider the following contrasts of the cohort cholesterol means in the population:

$$\begin{aligned}
\theta_3 &= (-1, 1, -1, 1)'\mu \\
\theta_4 &= (-1, -1, 1, 1)'\mu \\
\theta_5 &= (-1, 1, 1, -1)'\mu
\end{aligned}$$

Q: Are these contrasts orthogonal?

Q: True/False: $SS(\hat{\theta}_3) + SS(\hat{\theta}_4) + SS(\hat{\theta}_5) = SS[Trt]$

<u>Another exercise:</u>

1. Compute the sums of squares for the estimated contrasts in 3., 4. and 5. using the exercise just completed and the fact that if $\hat{\theta} = \sum c_i \bar{y}_{i+}$ then

$$SS[\hat{\theta}] = \frac{\hat{\theta}^2}{\sum \frac{c_i^2}{n_i}}.$$

2. For each $i = 3, 4, 5$, obtain the F-ratio to test $H_0 : \theta_i = 0$.
3. Critical value for each test? ________________. Draw conclusions.
   (3) an age effect
   (4) a gender effect
   (5) an age $\times$ gender interaction

Types of effects

Two-way ANOVA model for the cholesterol measurements:

$$\boxed{\begin{aligned} Y_{ijk} &= \\ i &= 1,2 = a \text{ and } j = 1,2 = b \text{ and } k = 1,2,\ldots,7 = n. \end{aligned}}$$

$E_{ijk} \overset{iid}{\sim} N(0,\sigma^2)$. Factors A, B: each with two levels ($a = b = 2$).

Three types of effects: ____________, ____________, ____________.

Exercise: Classify the contrasts from slide 4 as simple, interaction or main effects:

1. Estimate the mean difference in cholesterol between young men and young women.
2. Estimate the mean difference between old men and old women.
3. Estimate the mean difference between men and women.
4. Estimate the mean difference between older and younger folks.
5. Estimate the mean difference between the differences estimated in 1. and 2.

		GENDER		
		female ($j=1$)	male ($j=2$)	
AGE	younger ($i=1$)	194.7	209.4	
	older ($i=2$)	212.0	251.3	

Partitioning the treatment SS into $t-1$ orthogonal components

$$12281 = SS[Trt] = SS[\hat{\theta}_3] + SS[\hat{\theta}_4] + SS[\hat{\theta}_5] = 5103 + 6121 + 1056$$

SS and F test for interaction effect

$H_0 : (\alpha\beta)_{ij} \equiv 0$ (i.e. no interaction) versus $H_1 : (\alpha\beta)_{ij} \neq 0$ for some i, j.

$$\text{Use } \theta_5 = \mu(AB) \quad \text{and} \quad F = \frac{SS(\widehat{\theta}_{AB})/((a-1)(b-1))}{MS[E]}$$

on $df = 1, 24$. For cholesterol data the estimated interaction effect is

$$\widehat{\theta}_{AB} = \widehat{\theta}_5 = \widehat{\mu}(AB) = (251.3 - 209.4) - (212 - 194.7) = 41.9 - 17.3 = 24.6$$

the associated sum of squares is

$$SS(\hat{\theta}_5) = \frac{(24.6)^2}{\frac{1}{7} + \frac{(-1)^2}{7} + \frac{(-1)^2}{7} + \frac{1}{7}} = \frac{(24.6)^2}{\frac{4}{7}} = 1056$$

and $F = 1056/1185 = 0.9$ which (is/isn't) significant at $\alpha = 0.05$ on 1,24 df.
Conclusion: men's age effect (41.9) not significantly greater than women's (17.3)

F test for main effects

To test for main effect of A: AGE $H_0 : \alpha_1 = \alpha_2 = 0$ vs. $H_1 : \alpha_i$ not both 0

$$\text{use} \quad \theta_4 = \mu(A) \quad \text{and} \quad F = \frac{SS(\hat{\theta}_4)}{MS[E]}$$

on $1, 24$ df. The estimated main effect of AGE is

$$\hat{\mu}(A) = \frac{(251.3 - 209.4)}{2} + \frac{(212 - 194.7)}{2} = \frac{59.2}{2} = 29.6$$

the associated sum of squares is

$$SS(\hat{\theta}_4) = \frac{(29.6)^2}{\frac{(\frac{1}{2})^2}{7} + \frac{(\frac{1}{2})^2}{7} + \frac{(\frac{1}{2})^2}{7} + \frac{(\frac{1}{2})^2}{7}} = \frac{(29.6)^2}{\frac{1}{7}} = 6121$$

and

$$F = 6121/1185 = 5.2$$

Similarly for the main effect of B: gender

$$\hat{\mu}(B) = \frac{209.4 - 194.7}{2} + \frac{251.3 - 212}{2} = 27, SS(\widehat{\theta}_3) = 5103$$

Leading to $F_A = 6121/1185 = 5.2, F_B = 5103/1185 = 4.3$ since $F(0.05, \quad , \quad) = 4.26$ both GENDER and AGE effects significant at $\alpha = 0.05$.

Reminder: If SS[Model] on $df = t - 1$ can be partitioned into $t - 1$ orthogonal contrast sums of squares, then

$$SS[Trt] = \sum_{1}^{t-1} SS(\widehat{\theta}_i)$$

. For cholesterol,

$$\begin{aligned} SS[Trt] &= SS(\quad) + SS(\quad) + SS(\quad) \\ &= \qquad + \qquad + \end{aligned}$$

Confidence intervals for effects

If $\theta = c'\mu$, $100(1-\alpha)\%$ confidence interval given by

$$\boxed{\hat{\theta} \pm t(\alpha/2, N-t)\sqrt{MS[E]\sum \frac{c_i^2}{n_i}}}$$

For the cholesterol data, with $t(0.025, 24) = 2.06$ we have a 95% confidence interval for the AGE $\times$ GENDER interaction effect:

$$24.6 \pm 2.06\sqrt{\frac{4}{7}1185} \text{ or } 24.6 \pm 2.06(26.0) \text{ or } (-29, 78)$$

a 95% confidence interval for the AGE effect:

$$29.6 \pm 2.06\sqrt{\frac{1}{7}1185} \text{ or } 29.6 \pm 2.06(13.0) \text{ or } \text{ or } 29.6 \pm 26.8 \text{ or } (2.7, 56.4)$$

and a 95% confidence interval for the GENDER effect: ____________________

The term under the $\sqrt{}$ is the estimated standard error of the estimated contrast:

$$\widehat{SE}\left(\sum c_i \bar{y}_{i\cdot}\right) = \sqrt{MS[E]\sum \frac{c_i^2}{n_i}}$$

SAS code for cholesterol problem

```
data one;
   input cohort $ @;
   do subj=1 to 7;
      input y @;
      if cohort="I" then do; gender="W"; age="y"; end;
      else if cohort="II" then do; gender="M"; age="y";end;
      else if cohort="III" then do; gender="W" ; age="o";end;
      else if cohort="IV" then do; gender="M" ; age="o";end;
      output;
   end;
cards;
I    221   213   202   183   185   197   162
II    271   192   189   209   227   236   142
III    262   193   224   201   161   178   265
IV   192   253   248   278   232   267   289
;
run;
proc glm;
   class cohort;
   model y=cohort/clparm;
   constrast "main effect of age    " cohort -1 -1 1 1;
   constrast "main effect of gender" cohort -1 1 -1 1;
   constrast "interaction effect    " cohort -1 1 1 -1;
   estimate "main effect of age    " cohort -1 -1 1 1/divisor=2;
   estimate "main effect of gender" cohort -1 1 -1 1/divisor=2;
   estimate "interaction effect    " cohort -1 1 1 -1;
run;

proc glm;
   class gender age;
   model y=age|gender;
run;
```

(SAS will overlook misspelling of `contrast`.)

SAS output (abbreviated) for cholesterol problem

```
                              The SAS System                                    1
                             The GLM Procedure

                          Class Level Information
                    Class          Levels     Values
                    cohort              4     I II III IV

                                       Sum of
 Source                     DF        Squares    Mean Square  F Value   Pr > F
 Model                       3    12280.85714     4093.61905     3.46   0.0323
 Error                      24    28434.57143     1184.77381
 Corrected Total            27    40715.42857

           R-Square     Coeff Var      Root MSE        y Mean
           0.301627      15.87245      34.42054      216.8571

Contrast                    DF    Contrast SS    Mean Square  F Value   Pr > F
main effect of age           1    6121.285714    6121.285714     5.17   0.0323
main effect of gender        1    5103.000000    5103.000000     4.31   0.0488
interaction effect           1    1056.571429    1056.571429     0.89   0.3544

                                              Standard
 Parameter                    Estimate           Error    t Value    Pr > |t|
 main effect of age         29.5714286      13.0097426       2.27      0.0323
 main effect of gender      27.0000000      13.0097426       2.08      0.0488
 interaction effect        -24.5714286      26.0194851      -0.94      0.3544

            Parameter                  95% Confidence Limits
            main effect of age          2.7206396   56.4222175
            main effect of gender       0.1492111   53.8507889
            interaction effect        -78.2730065   29.1301493
```

```
                              The GLM Procedure

                       Class          Levels     Values
                       gender              2     M W
                       age                 2     o y

                                        Sum of
Source                        DF       Squares     Mean Square   F Value   Pr > F
Model                          3   12280.85714      4093.61905      3.46   0.0323
Error                         24   28434.57143      1184.77381
Corrected Total               27   40715.42857

           R-Square     Coeff Var      Root MSE       y Mean
           0.301627      15.87245      34.42054     216.8571

Source                        DF      Type I SS    Mean Square   F Value   Pr > F
age                            1    6121.285714    6121.285714      5.17   0.0323
gender                         1    5103.000000    5103.000000      4.31   0.0488
gender*age                     1    1056.571429    1056.571429      0.89   0.3544
```

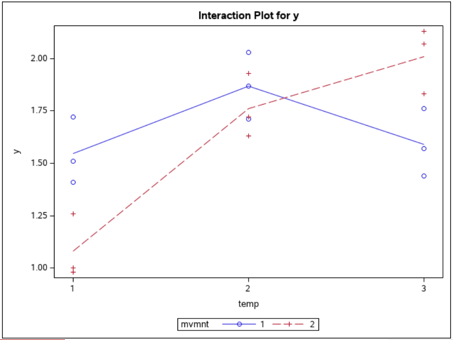
Exercise: (space on next page)

1. Express the effects below in terms of model parameters $\alpha_i, \beta_j, (\alpha\beta)_{ij}$:
   1. $\mu(AB_1)$ - __________ effect of __________ for __________ fixed at _____
   2. $\mu(AB_2)$ - simple effect of __________ for __________ fixed at _____
   3. $\mu(A_1B)$ - simple effect of __________ for __________ fixed at _____
   4. $\mu(A)$ - __________ effect of __________
   5. $\mu(B)$
   6. $\mu(AB)$ - difference of __________ and __________ (sometimes divided by two)
2. Estimate these effects

		GENDER	
		female ($j = 1$)	male ($j = 2$)
AGE	younger ($i = 1$)	194.7	209.4
	older ($i = 2$)	212.0	251.3

Weight gain by $N = 18$ tanks of fish randomized to $a \times b = 3 \times 2$ combinations of temperature and movement: (moodle: "`fishwtgain.dat`").

Temp	Movement	Tanks			mean
1	1	1.41	1.72	1.51	1.55
1	2	1.00	1.26	0.98	1.08
2	1	1.87	1.71	2.03	1.87
2	2	1.93	1.72	1.63	1.76
3	1	1.76	1.44	1.57	1.59
3	2	2.07	2.13	1.83	2.01



```
proc glm data=two;
   class temp mvmnt;
   model y=temp|mvmnt;
run;
```

```
The SAS System
The GLM Procedure

Class          Levels    Values
temp                3    1 2 3
mvmnt               2    1 2

                                      Sum of
Source                     DF        Squares   Mean Square  F Value  Pr > F
Model                       5     1.58689444    0.31737889    12.71  0.0002
Error                      12     0.29966667    0.02497222
Corrected Total            17     1.88656111

R-Square     Coeff Var      Root MSE        y Mean
0.841157      9.619440      0.158026      1.642778

Source                     DF      Type I SS   Mean Square  F Value  Pr > F
temp                        2     0.97747778    0.48873889    19.57  0.0002
mvmnt                       1     0.01227222    0.01227222     0.49  0.4967
temp*mvmnt                  2     0.59714444    0.29857222    11.96  0.0014

Source                     DF    Type III SS   Mean Square  F Value  Pr > F
temp                        2     0.97747778    0.48873889    19.57  0.0002
mvmnt                       1     0.01227222    0.01227222     0.49  0.4967
temp*mvmnt                  2     0.59714444    0.29857222    11.96  0.0014
```

An aside: only treatment totals and ANOVA table available in textbook, so data were synthesized:

```
data one;
   array ttotals{3,2} (4.65,3.24,5.61,5.28,4.77,6.03);
   do temp=1 to 3;
   do mvmnt=1 to 2;
      ymean=ttotals{temp,mvmnt}/3;
      do rep=1 to 3;
         error=rannor(123);
         output;
      end;
   end;
   end;
run;
proc standard data=one out=two mean=0 std=.1581139;
   by temp mvmnt ;
   var error;
run;
data two;
   set two;
   y=ymean+error;
   y=round(y,0.01);
run;
```

3×2 fish weight gain example continued

The effect of temperature (1=cold,2=lukewarm,3=warm) depends on movement:

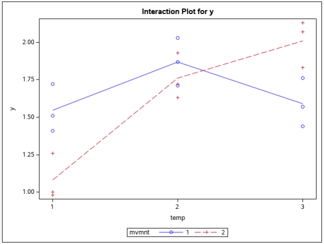
- when water still (mvmnt=1), temp effect is ______________
- when water moving (mvmnt=2), temp effect is ______________

The GLM Procedure

Level of temp	N	y Mean	y Std Dev
1	6	1.31333333	0.29173047
2	6	1.81500000	0.15280707
3	6	1.80000000	0.27085051

Level of mvmnt	N	y Mean	y Std Dev
1	9	1.66888889	0.20551426
2	9	1.61666667	0.43823510

Level of temp	Level of mvmnt	N	y Mean	y Std Dev
1	1	3	1.54666667	0.15821926
1	2	3	1.08000000	0.15620499
2	1	3	1.87000000	0.16000000
2	2	3	1.76000000	0.15394804
3	1	3	1.59000000	0.16093477
3	2	3	2.01000000	0.15874508



Partitioning $SS[Tot]$ in $a \times b$ design (Two-way ANOVA)

$$\boxed{Y_{ijk} \quad = \quad \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + E_{ijk}}$$
$$(i = 1, 2, \ldots, a \text{ and } j = 1, 2, \ldots, b \text{ and } k = 1, 2, \ldots, n)$$

Deviations:
total : $y_{ijk} - \overline{y}_{...}$
due to level i of factor A: ________________
due to level j of factor B: ________________
due to levels i of factor A and j of factor B after subtracting main effects:

$$\bar{y}_{ij.} - \bar{y}_{...} -$$

$$SS[Tot] = \sum_i \sum_j \sum_k (y_{ijk} - \qquad\qquad)^2 = \sum_i \sum_j \sum_k (\overline{y}_{ij.} - \qquad\qquad)^2 + \sum_i \sum_j \sum_k (y_{ijk} - \qquad\qquad)^2$$

$$SS[A] = \sum_i \sum_j \sum_k (\qquad\qquad\qquad)^2, \quad SS[B] = \sum_i \sum_j \sum_k (\qquad\qquad\qquad)^2$$

$$SS[AB] = \sum_i \sum_j \sum_k (\qquad\qquad\qquad\qquad\qquad)^2, SS[E] = \sum_i \sum_j \sum_k (\qquad\qquad\qquad)^2$$

ANOVA for two-factor crossed design

$$y_{ijk} = \bar{y}_{\cdot\cdot\cdot} + (\bar{y}_{i\cdot\cdot} - \bar{y}_{\cdot\cdot\cdot}) + (\bar{y}_{\cdot j\cdot} - \bar{y}_{\cdot\cdot\cdot}) + (\bar{y}_{ij\cdot} - \bar{y}_{i\cdot\cdot} - \bar{y}_{\cdot j\cdot} + \bar{y}_{\cdot\cdot\cdot}) + y_{ijk} - \bar{y}_{ij\cdot}$$

$$y_{ijk} - \bar{y}_{\cdot\cdot\cdot} = (\bar{y}_{i\cdot\cdot} - \bar{y}_{\cdot\cdot\cdot}) + (\bar{y}_{\cdot j\cdot} - \bar{y}_{\cdot\cdot\cdot}) + (\bar{y}_{ij\cdot} - \bar{y}_{i\cdot\cdot} - \bar{y}_{\cdot j\cdot} + \bar{y}_{\cdot\cdot\cdot}) + y_{ijk} - \bar{y}_{ij\cdot}$$

Square both sides, sum over i, j, k, and the $\times$-products vanish.

$$\begin{aligned} SS(Tot) &= SS(Trt) + SS(\qquad\qquad) \\ SS(Trt) &= SS(A) + \qquad\qquad + \end{aligned}$$

Analysis of replicated two (or more) factor designs often proceed according to the following steps:

1. Check for interaction
   1. If no interaction, analyze main effects
   2. If interaction, analyze simple effects

Test for interaction effect in 2×2 generalizes to $a \times b$:

$$H_0 : (\alpha\beta)_{ij} \equiv 0 \text{ vs. } H_1 : (\alpha\beta)_{ij} \neq 0 \text{ for some } i, j$$

$$F = \frac{MS[AB]}{MS[E]}$$

on $(a-1)(b-1)$ and $N - ab$ numerator, denominator df.

$$SS[AB] = n \sum_{i=1}^{3} \sum_{j=1}^{3} (\bar{y}_{ij\cdot} - \bar{y}_{i\cdot\cdot} - \bar{y}_{\cdot j\cdot} + \bar{y}_{\cdot\cdot\cdot})^2 = 0.597$$

$$F = \frac{.597/2}{0.025} = 11.96$$

which is highly significant ($p = 0.0014$) on 2,12 df.

We could proceed to test for main effects, but we won't.

Q: Why not?

A: Because effect of one factor depends on the level of the other factor, it might not make sense to talk about main effects.

If one insists on main effects, the appropriate F-ratios are

$$F_A = \frac{SS[A]/(a-1)}{MS[E]} \text{ on } a-1, N-ab \;\; df$$

$$F_B = \frac{SS[B]/(b-1)}{MS[E]} \text{ on } b-1, N-ab \;\; df$$

but the significance of the interaction effect suggests that the effect of one factor, say A, differs across levels of the other factor. A test for the main effect of A is based on the effect of A after *averaging over levels of B*. (Draw a picture.)

$a \times b$ designs

Yields on 36 tomato crops from balanced, complete, crossed design with $a = 3$ varieties (A) at $b = 4$ planting densities (B) :

Variety	Density $k/hectare$	Sample		
1	10	7.9	9.2	10.5
2	10	8.1	8.6	10.1
3	10	15.3	16.1	17.5
1	20	11.2	12.8	13.3
2	20	11.5	12.7	13.7
3	20	16.6	18.5	19.2
1	30	12.1	12.6	14.0
2	30	13.7	14.4	15.4
3	30	18.0	20.8	21.0
1	40	9.1	10.8	12.5
2	40	11.3	12.5	14.5
3	40	17.2	18.4	18.9

Statistical model?

$$Y_{ijk} =$$

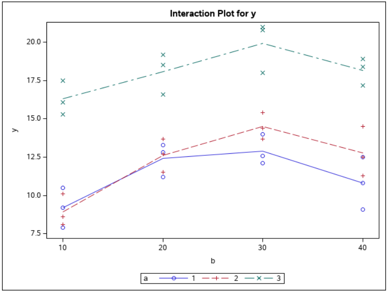
ANOVA table

```
                         The SAS System                                  1
                        The GLM Procedure

                Class          Levels    Values
                a                   3    1 2 3
                b                   4    10 20 30 40

                                     Sum of
Source                    DF        Squares    Mean Square   F Value   Pr > F

Model                     11    422.3155556     38.3923232     24.22   <.0001
Error                     24     38.0400000      1.5850000
Corrected Total           35    460.3555556

Source                    DF      Type I SS    Mean Square   F Value   Pr > F
a                          2    327.5972222    163.7986111    103.34   <.0001
b                          3     86.6866667     28.8955556     18.23   <.0001
a*b                        6      8.0316667      1.3386111      0.84   0.5484
```

Level of a	N	y Mean	y Std Dev
1	12	11.3333333	1.88309867
2	12	12.2083333	2.34887142
3	12	18.1250000	1.73369023

Level of b	N	y Mean	y Std Dev
10	9	11.4777778	3.75458978
20	9	14.3888889	2.96835158
30	9	15.7777778	3.36480972
40	9	13.9111111	3.53250777

Level of a	Level of b	N	y Mean	y Std Dev
1	10	3	9.2000000	1.30000000
1	20	3	12.4333333	1.09696551
1	30	3	12.9000000	0.98488578
1	40	3	10.8000000	1.70000000
2	10	3	8.9333333	1.04083300
2	20	3	12.6333333	1.10151411
2	30	3	14.5000000	0.85440037
2	40	3	12.7666667	1.61658075
3	10	3	16.3000000	1.11355287
3	20	3	18.1000000	1.34536240
3	30	3	19.9333333	1.67729942
3	40	3	18.1666667	0.87368949



A conventional look at main effects is just to make pairwise comparisons among marginal means, after averaging over other factors. Pairwise comparisons of density means using Tukey's procedure with $\alpha = 0.05$ are given below. (Use `means b/tukey;`. to obtain the output.)

```
                             The GLM Procedure

              Tukey's Studentized Range (HSD) Test for y

 NOTE: This test controls the Type I experimentwise error rate, but it
          generally has a higher Type II error rate than REGWQ.

             Alpha                                     0.05
             Error Degrees of Freedom                    24
             Error Mean Square                        1.585
             Critical Value of Studentized Range    3.90126
             Minimum Significant Difference          1.6372


       Means with the same letter are not significantly different.


          Tukey Grouping          Mean       N     b

                    A       15.7778       9     30
                    A
               B    A       14.3889       9     20
               B
               B            13.9111       9     40

                    C       11.4778       9     10
```

A three-factor example

In a balanced, complete, crossed design, $N = 36$ shrimp were randomized to $abc = 12$ treatment combinations from the factors below:

A1: Temperature at 25^o C
A2: Temperature at 35^o C
B1: Density of shrimp population at 80 shrimp/40l
B2: Density of shrimp population at 160 shrimp/40l
C1: Salinity at 10 units
C2: Salinity at 25 units
C3: Salinity at 40 units

The response variable of interest is weight gain Y_{ijkl} after four weeks.

Three-way ANOVA Model:

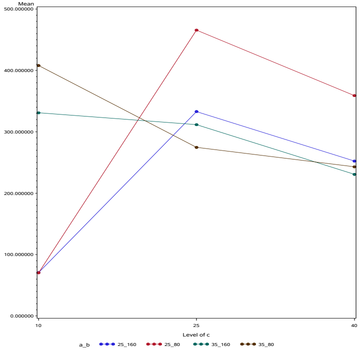
$$Y_{ijkl} =$$

$$i = 1,2 \quad j = 1,2 \quad k = 1,2,3 \quad l = 1,2,3$$

$$E_{ijkl} \overset{iid}{\sim} N(0, \sigma^2)$$

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	11	467636.3333	42512.3939	14.64	<.0001	
Error	24	69690.6667	2903.7778			sqrt(2MS(E)/3) ~= 44
Corrected Total	35	537327.0000				

Source	DF	Type I SS	Mean Square	F Value	Pr > F
a	1	15376.0000	15376.0000	5.30	0.0304
b	1	21218.7778	21218.7778	7.31	0.0124
a*b	1	8711.1111	8711.1111	3.00	0.0961
c	2	96762.5000	48381.2500	16.66	<.0001
a*c	2	300855.1667	150427.5833	51.80	<.0001
b*c	2	674.3889	337.1944	0.12	0.8909
a*b*c	2	24038.3889	12019.1944	4.14	0.0285



Level of a	Level of b	N	y Mean	y Std Dev
25	80	9	298.333333	185.106051
25	160	9	218.666667	128.739077
35	80	9	308.555556	85.475305
35	160	9	291.111111	57.953525

Level of a	Level of c	N	y Mean	y Std Dev
25	10	6	70.500000	15.109600
25	25	6	399.333333	114.206246
25	40	6	305.666667	69.987618
35	10	6	369.500000	56.450864
35	25	6	293.166667	45.375838
35	40	6	236.833333	38.096807

Level of b	Level of c	N	y Mean	y Std Dev
80	10	6	239.166667	188.065326
80	25	6	370.166667	122.218520
80	40	6	301.000000	77.415761
160	10	6	200.833333	144.240655
160	25	6	322.333333	74.529636
160	40	6	241.500000	32.788718

Level of a	Level of b	Level of c	N	y Mean	y Std Dev
25	80	10	3	70.333333	17.156146
25	80	25	3	465.666667	87.648921
25	80	40	3	359.000000	59.858166
25	160	10	3	70.666667	16.623277
25	160	25	3	333.000000	108.282039
25	160	40	3	252.333333	11.372481
35	80	10	3	408.000000	51.117512
35	80	25	3	274.666667	47.961790
35	80	40	3	243.000000	36.166283
35	160	10	3	331.000000	30.116441
35	160	25	3	311.666667	42.665365
35	160	40	3	230.666667	46.971623

$\boxed{1^{st} \text{ order}}$ interaction is between two factors
$\boxed{2^{nd} \text{ order}}$ interaction is between three factors

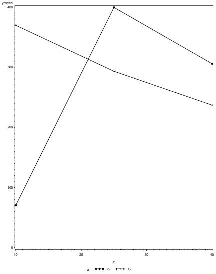
Upon inspection of the interaction plot, what do you see?
What is the primary two-factor/first-order interaction? ______________
Consider the means for low temperature (red and blue). Do you see evidence of BC interaction for temperature is low? Characterize it.
Do you see evidence of BC interaction for temperature is high?

If there is a BC interaction at one level of A but not the other, this is a second-order interaction.

Characterization of a three-factor interaction may not be unique. Here we first fixed A, but another analyst might first fix some other factor and characterize factorial effects in a different order.

```
%let d=divisor;   *an example of a macro variable;
data one;
   drop i;            /* a=temp, b=density, c=salinity */
   input a b c @;     * @ hold the line. prevent DATA step from loading ;
   do i=1 to 3;       *   new record when next INPUT encountered;
      input y @;      * @ hold the line;   *love isn't always on time! (Toto) ;
      y0=sqrt(y);
      output;
   end;
   cards;
   25 80 10 86 52 73
   25 80 25 544 371 482
   25 80 40 390 290 397
   25 160 10 53 73 86
   25 160 25 393 398 208
   25 160 40 249 265 243
   35 80 10 439 436 349
   35 80 25 249 245 330
   35 80 40 247 277 205
   35 160 10 324 305 364
   35 160 25 352 267 316
   35 160 40 188 223 281
;
run;

proc glimmix data=one;
   class a b c;
   model y=a|b|c;
   lsmeans a*b*c/slicediff=a*c;
run;
```

```
proc glimmix data=one;
   class a b c;
   model y=a|b|c;
   estimate "temp effect at c=1" a -1 1 a*c  -1 0 0   1 0 0;
   estimate "temp effect at c=2" a -1 1 a*c  0 -1 0   0 1 0;
   estimate "temp effect at c=3" a -1 1 a*c  0 0 -1   0 0 1;
   estimate "avg of temp effects at c=2,3" a -2 2  a*c 0 -1 -1  0 1 1/&d=2;
   estimate "mu[AC1]-.5(mu[AC2]+mu[AC3]) " a*c -2 1 1  2 -1 -1/divisor=2;
run;
```

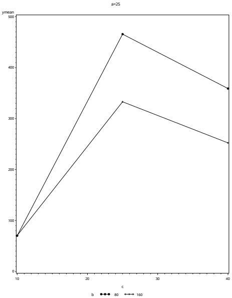


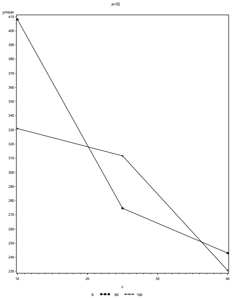
Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
temp effect at c=1	299.00	31.1115	24	9.61	<.0001
temp effect at c=2	-106.17	31.1115	24	-3.41	0.0023
temp effect at c=3	-68.8333	31.1115	24	-2.21	0.0367
avg of temp effects at c=2,3	-87.5000	21.9992	24	-3.98	0.0006
mu[AC1]-.5(mu[AC2]+mu[AC3])	386.50	38.1037	24	10.14	<.0001

We've characterized the $A \times C$ interaction. Note $SS(AC)$.

What else? The $B \times C$ interaction for fixed A.





$$
\begin{aligned}
\mu[A_1BC_1] &= \mu_{121} - \mu_{111} \\
\mu[A_1BC_2] &= \mu_{122} - \mu_{112} \\
\mu[A_1BC_3] &= \mu_{123} - \mu_{113} \\
\mu[A_2BC_1] &= \mu_{221} - \mu_{211} \\
\mu[A_2BC_2] &= \mu_{222} - \mu_{212} \\
\mu[A_2BC_3] &= \mu_{223} - \mu_{213}
\end{aligned}
$$

We can see now why it might not make sense to test for the "main effect" of B. The effect of B appears to be quite different across levels of A and C.
We can look at the effects of B after fixing A and C. For example,

$$\mu[A_1BC_1] = \beta_2 - \beta_1 + (\alpha\beta)_{12} - (\alpha\beta)_{11} + (\beta\gamma)_{21} - (\beta\gamma)_{11} + (\alpha\beta\gamma)_{121} - (\alpha\beta\gamma)_{111}$$

```
*class a b c;
estimate "density effect at c=1,a=1"
    b -1 1 a*b -1 1 b*c  -1 0 0   1 0 0 a*b*c  -1 0 0  1 0 0 ;
```

Similarly, we can look at lots of contrasts and compute contrast sums of squares:

```
proc glm data=one;
   class a b c;
   model y=a|b|c;
   estimate "density effect at c=1,a=1"
             b -1 1 a*b -1 1 b*c  -1 0 0   1 0 0 a*b*c  -1 0 0  1 0 0 ;
   estimate "density effect at c=2,a=1" b -1 1 b*c  0 -1 0   0 1 0
             b -1 1 a*b -1 1 b*c  0 -1 0   0 1 0 a*b*c  0 -1 0  0 1 0 ;
   estimate "density effect at c=3,a=1" b -1 1 b*c  0 0 -1   0 0 1
             b -1 1 a*b -1 1 b*c  0 0 -1   0 0 1 a*b*c  0 0 -1  0 0 1 ;
   estimate "mu[A1BC1]-.5(mu[A1BC2]+mu[A1BC3])" b*c  -2 1 1  2 -1 -1
            a*b*c -2 1 1  2 -1 -1/divisor=2;
   estimate "mu[A2BC1]-.5(mu[A2BC2]+mu[A2BC3])" b*c  -2 1 1  2 -1 -1
            a*b*c 0 0 0  0 0 0  -2 1 1  2 -1 -1/divisor=2;
   estimate "mu[A1BC1.....] - mu[A2BC1.....]"
            a*b*c -2 1 1  2 -1 -1  2 -1 -1 -2 1 1/divisor=2;

   contrast "density effect at c=1,a=1"
             b -1 1 a*b -1 1 b*c  -1 0 0   1 0 0 a*b*c  -1 0 0  1 0 0 ;
   contrast "density effect at c=2,a=1" b -1 1 b*c  0 -1 0   0 1 0
             b -1 1 a*b -1 1 b*c  0 -1 0   0 1 0 a*b*c  0 -1 0  0 1 0 ;
   contrast "density effect at c=3,a=1" b -1 1 b*c  0 0 -1   0 0 1
             b -1 1 a*b -1 1 b*c  0 0 -1   0 0 1 a*b*c  0 0 -1  0 0 1 ;
   contrast "mu[A1BC1]-.5(mu[A1BC2]+mu[A1BC3])" b*c  -2 1 1  2 -1 -1
            a*b*c -2 1 1  2 -1 -1;
   contrast "mu[A2BC1]-.5(mu[A2BC2]+mu[A2BC3])" b*c  -2 1 1  2 -1 -1
            a*b*c 0 0 0  0 0 0  -2 1 1  2 -1 -1;
   contrast "diff between last two"
            a*b*c -2 1 1  2 -1 -1  2 -1 -1 -2 1 1;
run;
```

Parameter	Estimate	Standard Error	t Value	Pr > |t|
density effect at c=1,a=1	0.333333	43.9983165	0.01	0.9940
density effect at c=2,a=1	-132.666667	43.9983165	-3.02	0.0060
density effect at c=3,a=1	-106.666667	43.9983165	-2.42	0.0232
mu[A1BC1]-.5(mu[A1BC2]+mu[A1BC3])	120.000000	53.8867124	2.23	0.0356
mu[A2BC1]-.5(mu[A2BC2]+mu[A2BC3])	-89.333333	53.8867124	-1.66	0.1104
mu[A1BC1.....] - mu[A2BC1.....]	209.333333	76.2073196	2.75	0.0112

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
mu[AC1]-.5(mu[AC2]+mu[AC3])	1	298764.5000	298764.5000	102.89	<.0001
density effect at c=1,a=1	1	0.1667	0.1667	0.00	0.9940
density effect at c=2,a=1	1	26400.6667	26400.6667	9.09	0.0060
density effect at c=3,a=1	1	17066.6667	17066.6667	5.88	0.0232
mu[A1BC1]-.5(mu[A1BC2]+mu[A1BC3])	1	14400.0000	14400.0000	4.96	0.0356
mu[A2BC1]-.5(mu[A2BC2]+mu[A2BC3])	1	7980.4444	7980.4444	2.75	0.1104
diff between last two	1	21910.2222	21910.2222	7.55	0.0112

a*b*c Effect Sliced by a*c for y

a	c	DF	Sum of Squares	Mean Square	F Value	Pr > F
25	10	1	0.166667	0.166667	0.00	0.9940
25	25	1	26401	26401	9.09	0.0060
25	40	1	17067	17067	5.88	0.0232
35	10	1	8893.500000	8893.500000	3.06	0.0929
35	25	1	2053.500000	2053.500000	0.71	0.4087
35	40	1	228.166667	228.166667	0.08	0.7816

Recall, $SS(AC) \sim 301000$ and $SS(ABC) \sim 24000$. We've explained most of this variation with two single df contrasts.

Activity w/ ESTIMATE statement

Exercise: identify the estimable contrasts in each of the ESTIMATE statements in the correspondence below, which pertains to a 3×2 study with factors and levels

Factor	Levels
A : additive	acetic, nothing, sorbate
B : uv	0,1.

```
To: osborne@stat.ncsu.edu
Subject: non estimatable estimate statements

I am still having trouble with the estimate statements, the only ones
that work for the additive*uv interaction are where we contrast the
same additive over the uv, can anything be done about this??

proc glm;
   class additive uv;
   model  ycount=additive uv uv*additive;
estimate 'acetic uv=0 vs acetic uv=1' uv  1 -1 uv*additive 1 -1 0 0 0 0;
estimate 'acetic uv=0 vs nothing uv=0' uv 1 -1 uv*additive 1 0 -1 0 0 0;
estimate 'acetic uv=0 vs nothing uv=1' uv 1 -1 uv*additive 1 0 0 -1 0 0;
estimate 'acetic uv=0 vs sorbate uv=0' uv 1 -1 uv*additive 1 0 0 0 -1 0;
estimate 'acetic uv=0 vs sorbate uv=1' uv 1 -1 uv*additive 1 0 0 0 0 -1;
estimate 'acetic uv=1 vs nothing uv=0' uv 1 -1 uv*additive 0 1 -1 0 0 0;
estimate 'acetic uv=1 vs nothing uv=1' uv 1 -1 uv*additive 0 1 0 -1 0 0;
estimate 'acetic uv=1 vs sorbate uv=0' uv 1 -1 uv*additive 0 1 0 0 -1 0;
estimate 'acetic uv=1 vs sorbate uv=1' uv 1 -1 uv*additive 0 1 0 0 0 -1;
estimate 'nothing uv=0 vs nothing uv=1' uv 1 -1 uv*additive 0 0 1 -1 0 0;
estimate 'nothing uv=0 vs sorbate uv=0' uv 1 -1 uv*additive 0 0 1 0 -1 0;
estimate 'nothing uv=0 vs sorbate uv=1' uv 1 -1 uv*additive 0 0 1 0 0 -1;
estimate 'nothing uv=1 vs sorbate uv=0' uv 1 -1 uv*additive 0 0 0 1 -1 0;
estimate 'nothing uv=1 vs sorbate uv=1' uv 1 -1 uv*additive 0 0 0 1 0 -1;
estimate 'sorbate uv=0 vs sorbate uv=1'uv 1 -1 uv*additive 0 0 0 0 1 -1;
estimate 'uv=0 vs uv=1' uv 1 -1;
estimate 'acetic vs nothing' additive 1 -1;
estimate 'acetic vs sorbate' additive 1 0 -1;
estimate 'nothing vs sorbate' additive 0 1 -1;
```

Unbalanced design

Recall the 2×2 cholesterol study. Suppose the study is unbalanced:

	Gender		
Age	Male	Female	Marginal mean
young	271,192,189,209, 227,236	162	$\bar{y}_{1++} = 212.3$
old	289	262,193,224,201 161,178,265	$\bar{y}_{2++} = 221.6$
	$\bar{y}_{+1+} = 230.4$	$\bar{y}_{+2+} = 205.8$	

$$\bar{y}_{11} = 220.7, \ \bar{y}_{12} = 162, \ \bar{y}_{21} = 289, \ \bar{y}_{22} = 212.$$

Consider an additive two-factor ANOVA model: $Y_{ijk} = \mu + \alpha_i + \beta_j + E_{ijk}$
Exercise: finish parametric expressions for expected values below:

$$\begin{aligned} E(\bar{Y}_{1++}) &= \mu + \alpha_1 + \frac{1}{7}(6\beta_1 + \beta_2) \\ E(\bar{Y}_{2++}) &= \mu + \alpha_2 + \frac{1}{8}(\beta_1 + 7\beta_2) \\ E(\bar{Y}_{+1+}) &= \\ E(\bar{Y}_{+2+}) &= \end{aligned}$$

Marginal sample means are not real useful in this unbalanced study.

Least squares means: (what would be estimated by marginal means if design *were* balanced).

Parametric expressions for population means given below for additive model:

Population group	effect of interest	estimate
Young folks	$\mu + \alpha_1 + \frac{1}{2}(\beta_1 + \beta_2)$	188.03
Older folks	$\mu + \alpha_2 + \frac{1}{2}(\beta_1 + \beta_2)$	
Men	$\mu + \frac{1}{2}(\alpha_1 + \alpha_2) + \beta_1$	
Women	$\mu + \frac{1}{2}(\alpha_1 + \alpha_2) + \beta_2$	
Young men	$\mu + \alpha_1 + \beta_1$	
Older men	$\mu + \alpha_2 + \beta_1$	
Young women	$\mu + \alpha_1 + \beta_2$	
Older women	$\mu + \alpha_2 + \beta_2$	

`lsmeans age gender;` will report least squares estimates for first four means above.

```
                                          Standard
gender              y LSMEAN                 Error     Pr > |t|
m                 251.525773             16.233482       <.0001
w                 183.597938             15.842256       <.0001

                                          Standard
  age               y LSMEAN                 Error     Pr > |t|
  jr              188.025773             16.233482       <.0001
  sr              247.097938             15.842256       <.0001
```

These quantities estimated using linear combinations of the treatment means of the form:

$$\hat{\theta} = c_{11}\bar{y}_{11+} + c_{12}\bar{y}_{12+} + c_{21}\bar{y}_{21+} + c_{22}\bar{y}_{22+}.$$

The coefficients are chosen so that $E(\hat{\theta}) = \theta$ and $\sum c_{ij}^2/n_{ij}$ is minimized.

Example: What are the coefficients for the contrast which estimates the population mean for young folks, $\mu + \alpha_1 + \frac{1}{2}(\beta_1 + \beta_2)$ with minimum variance?

$$\begin{aligned} c_{11} + c_{12} &= 1(\text{coeff for } \alpha_1) \\ c_{21} + c_{22} &= 0(\text{coeff for } \alpha_2) \\ c_{11} + c_{21} &= \frac{1}{2}(\text{coeff for } \beta_1) \\ c_{12} + c_{22} &= \frac{1}{2}(\text{coeff for } \beta_2) \end{aligned}$$

Variance is then proportional to

$$\frac{c_{11}^2}{n_{11}} + \frac{c_{12}^2}{n_{12}} + \frac{c_{21}^2}{n_{21}} + \frac{c_{22}^2}{n_{22}} = \frac{c_{11}^2}{n_{11}} + \frac{(1 - c_{11})^2}{n_{12}} + \frac{(\frac{1}{2} - c_{11})^2}{n_{21}} + \frac{(c_{11} - \frac{1}{2})^2}{n_{22}}$$

minimized at $c_{11} = \frac{66}{97}$ by setting derivative to 0, solving. LS mean then

$$\hat{\theta} = \frac{66}{97}\bar{y}_{11+} + (1 - \frac{66}{97})\bar{y}_{12+} + (\frac{1}{2} - \frac{66}{97})\bar{y}_{21+} + (\frac{66}{97} - \frac{1}{2})\bar{y}_{22+} = 188.03.$$

Similarly for old folks, the contrast with minimum variance has

$$c_{11} = 18/97 = -c_{12}$$

and

$$c_{21} = \frac{1}{2} - 18/97, c_{22} = \frac{1}{2} + 18/97$$

so that the estimate for the old folks mean is

$$\frac{18}{97}\bar{y}_{11+} - \frac{18}{97}\bar{y}_{12+} + (\frac{1}{2} - \frac{18}{97})\bar{y}_{21+} + (\frac{1}{2} + \frac{18}{97})\bar{y}_{22+} = 247.1.$$

Exercise: obtain least squares estimators and estimates of marginal means for men and women as well as for each age×gender combination.

Q: Is there an age effect? Should we base our conclusion on

$$\sum_i \sum_j \sum_k (\bar{y}_{i++} - \bar{y}_{+++})^2 = 325.6?$$

A: Might not be a good idea if factor B has an effect.
(This is the unadjusted sum of squares for age.)
Alternatively, consider the contrast

$$\theta_{age} = \alpha_1 - \alpha_2.$$

We can obtain the coefficients of the LS estimate of this contrast and then use them to get sum of squares for age effect adjusted for gender, or type II SS

$$\hat{\theta}_{age} = \hat{\alpha}_1 - \hat{\alpha}_2 = c_{11}\bar{y}_{11+} + c_{12}\bar{y}_{12+} + c_{21}\bar{y}_{21+} + c_{22}\bar{y}_{22+} \qquad \text{where}$$

$$\begin{aligned} c_{11} + c_{12} &= 1(\text{coeff for } \alpha_1) \\ c_{21} + c_{22} &= -1(\text{coeff for } \alpha_2) \\ c_{11} + c_{21} &= 0(\text{coeff for } \beta_1) \\ c_{12} + c_{22} &= 0(\text{coeff for } \beta_2) \end{aligned}$$

$\text{Var}(\hat{\theta}_{age})$ is minimized when $c_{11} = \frac{48}{97}$ which leads to

$$\hat{\theta}_{age} = -59.07$$

with

$$SS[\hat{\theta}_{age}] = \frac{(-59.07)^2}{\frac{(\frac{48}{97})^2}{6} + \frac{(-1-\frac{48}{97})^2}{1} + \frac{(\frac{-48}{97})^2}{1} + \frac{(-1+\frac{48}{97})^2}{7}} = 6044.$$

```
/*
I   221  213  202  183  185  197  162
II  271  192  189  209  227  236  142
III 262  193  224  201  161  178  265
IV  192  253  248  278  232  267  289
*/
options ls=75;
data one;
   input gender $ age $2. @;
   do i=1 to 7;
      input y @@;
      output;
   end;
cards;
w jr  .  .  .  .  .  .  162
m jr  271  192  189  209  227  236  .
w sr  262  193  224  201  161  178  265
m sr  .  .  .  .  .  .  289
;
run;

proc glm;
   class age gender;
   model y=age gender/solution;
   lsmeans gender age/stderr;
   estimate "lsmean for young folks" intercept 2 age 2 0 gender 1 1/divisor=2;
   estimate "lsmean for older folks" intercept 2 age 0 2 gender 1 1/divisor=2;
   estimate "lsmean for men" intercept 2 age 1 1 gender 2 0/divisor=2;
   estimate "lsmean for women" intercept 2 age 1 1 gender 0 2/divisor=2;
   estimate "lsmean for young men" intercept 1 age 1 0 gender 1 0;
   estimate "lsmean for young women" intercept 1 age 1 0 gender 0 1;
   estimate "lsmean for old men" intercept 1 age 0 1 gender 1 0;
   estimate "lsmean for old women" intercept 1 age 0 1 gender 0 1;
   contrast "age effect" age 1 -1;
   estimate "age effect" age 1 -1;
   contrast "gender effect" gender 1 -1;
   means gender age;
run;
```

```
                              The SAS System                                   1
                             The GLM Procedure
                         Class Level Information

                      Class          Levels    Values
                      age                 2    jr sr
                      gender              2    m w

                       Number of observations    28

NOTE: Due to missing values, only 15 observations can be used in this
      analysis.

                                       Sum of
 Source                     DF        Squares    Mean Square  F Value   Pr > F
 Model                       2     8318.06735     4159.03368     3.42   0.0669
 Error                      12    14606.86598     1217.23883
 Corrected Total            14    22924.93333

            R-Square     Coeff Var      Root MSE        y Mean
            0.362839      16.05812      34.88895      217.2667

 Source                     DF      Type I SS    Mean Square  F Value   Pr > F
 age                         1     325.629762     325.629762     0.27   0.6144
 gender                      1    7992.437592    7992.437592     6.57   0.0249

 Source                     DF    Type III SS    Mean Square  F Value   Pr > F
 age                         1    6044.348306    6044.348306     4.97   0.0457
 gender                      1    7992.437592    7992.437592     6.57   0.0249

                                           Standard
  Parameter             Estimate              Error    t Value    Pr > |t|
  Intercept          213.1340206 B      12.77243521      16.69      <.0001
  age       jr       -59.0721649 B      26.50916484      -2.23      0.0457
  age       sr         0.0000000 B        .                .         .
  gender    m         67.9278351 B      26.50916484       2.56      0.0249
  gender    w          0.0000000 B        .                .         .
```

```
                      Least Squares Means

                                   Standard
         gender         y LSMEAN      Error    Pr > |t|
         m            251.525773   16.233482     <.0001
         w            183.597938   15.842256     <.0001

                                  Standard
           age         y LSMEAN      Error    Pr > |t|
           jr        188.025773   16.233482     <.0001
           sr        247.097938   15.842256     <.0001

Contrast                 DF    Contrast SS    Mean Square   F Value   Pr > F
age effect                1    6044.348306    6044.348306      4.97   0.0457
gender effect             1    7992.432592    7992.437592      6.57   0.0249

                                             Standard
Parameter                      Estimate         Error    t Value     Pr > |t|
lsmean for young folks       188.025773    16.2334818      11.58       <.0001
lsmean for older folks       247.097938    15.8422561      15.60       <.0001
lsmean for men               251.525773    16.2334818      15.49       <.0001
lsmean for women             183.597938    15.8422561      11.59       <.0001
lsmean for young men         221.989691    13.7197962      16.18       <.0001
lsmean for young women       154.061856    26.2714097       5.86       <.0001
lsmean for old men           281.061856    26.2714097      10.70       <.0001
lsmean for old women         213.134021    12.7724352      16.69       <.0001
age effect                   -59.072165    26.5091648      -2.23       0.0457
```