*Topics for these notes*:

- *Estimation in the general linear model.*
  - *Estimation in specific models*
  - *Time as class versus continuous*
  - *Estimability*
  - *Properties of 'good' estimators*
  - *Standard errors and confidence intervals*

*Associated reading: Section 4 in 'General linear models' course notes.*

# *4 Estimation*

## *4.1 Computing estimates – methods and application*

### *4.1.1 Myostatin data in a one-way effects model*

The Myostatin data came from a 2×3 factorial treatment structure in a CRD, and hence was analyzed with a 2-way ANOVA. We can also fit it using the one-way effects model, where there are 2×3 = 6 levels of this new 'composite' factor. This is considered next.

Write the vectors $\mathbf{Y}$ and $\boldsymbol{\beta}$ and matrix $\mathbf{X}$ associated with this data, in the one-way effects model:

$$\mathbf{Y} = (Y_{11} \;\; Y_{12} \;\; Y_{13} \;\; Y_{14} \;\; Y_{21} \ldots Y_{61} \;\; Y_{62} \;\; Y_{63} \;\; Y_{64})^t$$

$$\boldsymbol{\beta} = (\mu \;\; \kappa_1 \;\; \kappa_2 \;\; \kappa_3 \;\; \kappa_4 \;\; \kappa_5 \;\; \kappa_6)^t$$

$$\mathbf{X} = \begin{pmatrix} 1 & 1 & 0 & 0 & \ldots & 0 \\ 1 & 1 & 0 & 0 & \ldots & 0 \\ 1 & 1 & 0 & 0 & \ldots & 0 \\ 1 & 1 & 0 & 0 & \ldots & 0 \\ 1 & 0 & 1 & 0 & \ldots & 0 \\ 1 & 0 & 1 & 0 & \ldots & 0 \\ \ldots & \ldots & \ldots & \ldots & \ldots & \ldots \\ 1 & 0 & 0 & 0 & \ldots & 1 \\ 1 & 0 & 0 & 0 & \ldots & 1 \\ 1 & 0 & 0 & 0 & \ldots & 1 \\ 1 & 0 & 0 & 0 & \ldots & 1 \end{pmatrix}$$

Note that $\mathbf{X}$ does not have full rank (e.g., first column is the sum of the next 6). Thus, $(\mathbf{X}'\mathbf{X})^{-1}$ does not exist. We'll need to use a generalized inverse.

# The fit of this model with the data application can be obtained as follows:

```
*One-way version;
proc glm data=myostatin; class group time; model y = group*time / solution; run;
```

```
The GLM Procedure
Dependent Variable: y

                                 Sum of
Source                     DF       Squares     Mean Square    F Value    Pr > F
Model                       5    23.12640221      4.62528044       8.02    0.0004
Error                      18    10.37454375      0.57636354
Corrected Total            23    33.50094596

      R-Square     Coeff Var      Root MSE        y Mean
      0.690321      14.04979      0.759186      5.403542

                                                Standard
Parameter                 Estimate                 Error    t Value    Pr > |t|
Intercept            3.777750000 B      0.37959305       9.95      <.0001
group*time c 24      3.184250000 B      0.53682564       5.93      <.0001
group*time c 48      1.673000000 B      0.53682564       3.12      0.0060
group*time c 72      1.133500000 B      0.53682564       2.11      0.0490
group*time m 24      2.290500000 B      0.53682564       4.27      0.0005
group*time m 48      1.473500000 B      0.53682564       2.74      0.0133
group*time m 72      0.000000000 B       .                .         .

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the
normal equations.  Terms whose estimates are followed by the letter 'B' are not uniquely estimable.
```

- A least squares solution to $\boldsymbol{\beta} = (\mu \ \ \kappa_1 \ \kappa_2 \ \kappa_3 \ \kappa_4 \ \kappa_5 \ \kappa_6)^t$ for the Myostatin data is given in the "Parameter estimates" of the previous SAS output. To solve for $\hat{\boldsymbol{\beta}}$, SAS uses a generalized inverse for $\mathbf{X}^t\mathbf{X}$ due to the linear dependency issue that we learned about in Section 3 ($\mathbf{X}$ and thus $\mathbf{X}^t\mathbf{X}$ do not have full rank).

- The fact that $\mathbf{X}$ is not of full rank is easy to see, since the first column of $\mathbf{X}$ is the sum of the other columns, i.e., the columns are not linearly independent. SAS uses the generalized inverse for $\mathbf{X}^t\mathbf{X}$ that is equivalent to setting the highest level of *group*×*time* to 0.

- Consequently, the $\hat{\boldsymbol{\beta}}$ solution is not unique (which relates to the NOTE at the end of the output). This is easy to see since we could have picked any other level to be the reference level, which would in turn alter the estimates. The 'NOTE' does not indicate an error; we just need to be careful about how to which functions of parameters to consider.

The PROC IML code below computes least squares estimates for **β**, for the one-way effects model involving the Myostatin data.

```
proc iml;
*The one-way ANOVA model - using the MP generalized inverse approach;
x={1 1 0 0 0 0 0, 1 1 0 0 0 0 0, 1 1 0 0 0 0 0, 1 1 0 0 0 0 0,
   1 0 1 0 0 0 0, 1 0 1 0 0 0 0, 1 0 1 0 0 0 0, 1 0 1 0 0 0 0,
   1 0 0 1 0 0 0, 1 0 0 1 0 0 0, 1 0 0 1 0 0 0, 1 0 0 1 0 0 0,
   1 0 0 0 1 0 0, 1 0 0 0 1 0 0, 1 0 0 0 1 0 0, 1 0 0 0 1 0 0,
   1 0 0 0 0 1 0, 1 0 0 0 0 1 0, 1 0 0 0 0 1 0, 1 0 0 0 0 1 0,
   1 0 0 0 0 0 1, 1 0 0 0 0 0 1, 1 0 0 0 0 0 1, 1 0 0 0 0 0 1};
y={6.568, 6.802, 7.198, 7.280, 4.992, 5.242, 5.285, 6.284,
   4.092, 4.331, 5.135, 6.087, 5.516, 6.023, 6.334, 6.400,
   4.512, 4.706, 5.175, 6.612, 3.076, 3.209, 3.462, 5.364};

xt=t(x);
xtx=xt*x;
xginv=ginv(x);
xtxginv=ginv(xtx);
px=x*xtxginv*xt;
pred_values=px*y;
betahat=xtxginv*xt*y;
```

Note: the 'ginv' function in R is the Moore-Penrose inverse, which is not the same as SAS's generalized inverse.

This is the projection matrix.

```
print
pred_values;

PRED_VALUES
6.962
6.962
…

3.77775
3.77775
```

```
print
betahat;

BETAHAT
4.6316071
2.3303929
0.8191429
0.2796429
1.4366429
0.6196429
-0.853857
```

Note that PROC GLM yielded a different solution due to the different g-inverse used. But estimates of $\mu + \kappa_i$ will be the same. E.g., the estimate of $\mu + \kappa_1$ =4.63+2.33 = 6.96 here; GLM in SAS approach: 3.78+3.18=6.96.

In the example above, the Moore-Penrose inverse was used. However, other approaches could be used to find generalized inverses. For SAS's default approach, what is the first column found to be linearly dependent on the others, moving from left to right?

Finally, here is the R fit with the one-way data. Differences are again due to use of reference group; R uses ‘Control at 24 hours’, the lowest level of each factor:

```
#1-way effects model
class_fit2=lm(y ~ gt,data=myostatin)
summary(class_fit2)

Call:  lm(formula = y ~ gt, data = myostatin)

Residuals:
    Min      1Q  Median      3Q     Max
-0.8193 -0.5470 -0.1629  0.2788  1.5862

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   6.9620     0.3796  18.341 4.27e-13 ***
gtc48        -1.5113     0.5368  -2.815  0.01146 *
gtc72        -2.0508     0.5368  -3.820  0.00125 **
gtm24        -0.8938     0.5368  -1.665  0.11325
gtm48        -1.7108     0.5368  -3.187  0.00511 **
gtm72        -3.1843     0.5368  -5.932 1.30e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.7592 on 18 degrees of freedom
Multiple R-squared: 0.6903,     Adjusted R-squared: 0.6043
F-statistic: 8.025 on 5 and 18 DF,  p-value: 0.0003960
```

## *4.1.2 Myostatin data in a two-way effects model*

Now we get back to the analytical model that is more consistent with the actual treatment structure. The output on page 2 relates to the estimates that we will be deriving now.

Model with interaction

Write **X**, **Y** and **β** (for the two-way effects model).

The model: $Y_{ijk} = \mu + \alpha_i + \tau_j + \gamma_{ij} + \varepsilon_{ijk}$

$$\mathbf{X} = \begin{pmatrix} 1 & 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 1 & 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 1 & 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 1 & 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ . & . & . & & & & & & & & & \\ 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \quad \mathbf{y} = \begin{pmatrix} y_{111} \\ y_{112} \\ y_{113} \\ y_{114} \\ y_{121} \\ y_{122} \\ y_{123} \\ y_{124} \\ \cdots \\ y_{231} \\ y_{232} \\ y_{233} \\ y_{234} \end{pmatrix} = \begin{pmatrix} 6568 \\ 6802 \\ 7198 \\ 7280 \\ 4992 \\ 5242 \\ 5285 \\ 6284 \\ \cdots \\ 3076 \\ 3209 \\ 3462 \\ 5364 \end{pmatrix} \quad \boldsymbol{\beta} = \begin{pmatrix} \mu \\ \alpha_1 \\ \alpha_2 \\ \tau_1 \\ \tau_2 \\ \tau_3 \\ \gamma_{11} \\ \gamma_{12} \\ \gamma_{13} \\ \gamma_{21} \\ \gamma_{22} \\ \gamma_{23} \end{pmatrix}$$

Once again, the **X** matrix does not have full rank (i.e., at least one column is a linear combination of the others), so we can't use a regular inverse of $\mathbf{X}^t\mathbf{X}$ in calculating Beta hat. We will let SAS compute the generalized inverse. Again, the way SAS computes the generalized inverse is equivalent to setting the highest levels of factors to 0 (including levels of interactions that involve the highest level of at least one of the factors). Here is a review of the two-way model fit from SAS PROC GLM:

```
proc glm data=myostatin; class group time; model y = group|time / solution; run;
Parameter                  Estimate        Std. Error    t Value    Pr > |t|
Intercept             3.777750000 B      0.37959305       9.95      <.0001
group       c         1.133500000 B      0.53682564       2.11      0.0490
group       m         0.000000000 B       .                .         .
time        24        2.290500000 B      0.53682564       4.27      0.0005
time        48        1.473500000 B      0.53682564       2.74      0.0133
time        72        0.000000000 B       .                .         .
group*time  c 24     -0.239750000 B      0.75918610      -0.32      0.7558
group*time  c 48     -0.934000000 B      0.75918610      -1.23      0.2344
group*time  c 72      0.000000000 B       .                .         .
group*time  m 24      0.000000000 B       .                .         .
group*time  m 48      0.000000000 B       .                .         .
group*time  m 72      0.000000000 B       .                .         .

NOTE: The X'X matrix has been found to be singular...
```

SAS's generalized inverse is only one possibility; choosing another one will yield different Beta estimates. Thus, we need to determine what functions of parameters are estimable (to be discussed soon).

An alternative approach is to define a full-rank model beforehand. To do this, we can employ SAS PROC REG, creating our own indicator variables. We can also use the LM function in R, as shown below. In this approach, I have not used the 'factor' function as shown previously, but rather, I have created my own indicators so that the results match those of SAS (in which the highest levels of factors are set to 0).

Fitting the model in R – in this case instead of using the factor function as before, I manually create dummy variables so that the estimates are the same as those obtained from SAS.

```
leucine =c(6568,6802,7198,7280,4992,5242,5285,6284,4092,4331,5135,6087,5516,6023,
   6334,6400,4512,4706,5175,6612,3076,3209,3462,5364)/1000;
group=c(1,1,1,1,1,1,1,1,1,1,1,1,0,0,0,0,0,0,0,0,0,0,0,0)
time=c(24,24,24,24,48,48,48,48,72,72,72,72,24,24,24,24,48,48,48,48,72,72,72,72)
time_24=c(1,1,1,1,0,0,0,0,0,0,0,0,1,1,1,1,0,0,0,0,0,0,0,0)
time_48=c(0,0,0,0,1,1,1,1,0,0,0,0,0,0,0,0,1,1,1,1,0,0,0,0)
class_fit=lm(leucine ~ group + time_24 + time_48 + group*time_24 + group*time_48)
summary(class_fit)
```

```
Coefficients:
               Estimate Std. Error t value Pr(>|t|)
(Intercept)     3.7778     0.3796   9.952 9.62e-09 ***
group           1.1335     0.5368   2.111 0.048975 *
time_24         2.2905     0.5368   4.267 0.000464 ***
time_48         1.4735     0.5368   2.745 0.013318 *
group:time_24  -0.2397     0.7592  -0.316 0.755788
group:time_48  -0.9340     0.7592  -1.230 0.234435
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.7592 on 18 degrees of freedom
Multiple R-squared: 0.6903,     Adjusted R-squared: 0.6043
F-statistic: 8.025 on 5 and 18 DF,  p-value: 0.0003960
```

The beta estimates are the same as with the ‘less-than-full-rank’ approach since we used the highest levels of factors as reference points. We can also fit the model several different ways using PROC IML in SAS. See the Appendix in the GLM notes for detail.

Summary:

- In order to fit a two-way effects model, we need to deal with linear dependencies in **X**. In BIOS6612, we learned how to do this manually. Another approach is to let software do this automatically by using generalized inverses in the calculation. However, in the end, the fitted model will be the same when we consider functions of parameters that are uniquely estimable.

- In some cases, the two approaches will even yield the same individual beta estimates. For example, using the highest levels of factors as the reference group is equivalent to the way that SAS finds a generalized inverse.

## *4.1.3 Myostatin data in the means model*

If we simply remove the intercept from the one-way effects model, we have the means model. In SAS or R, you can remove the intercept easily. Below is the means model fit in SAS and R, plus some extra tests in R. Here, results are the same since the model already has full rank.

SAS code

```
proc glm
data=myostatin;
class group time;
model y = group*time
   / solution noint;
run;
```

SAS output

```
Parameter          Estimate   Std. Err.  t Value  Pr>|t|
group*time c 24     6.962      0.380     18.34     <.0001
group*time c 48     5.451      0.380     14.36     <.0001
group*time c 72     4.911      0.380     12.94     <.0001
group*time m 24     6.068      0.380     15.99     <.0001
group*time m 48     5.251      0.380     13.83     <.0001
group*time m 72     3.778      0.380      9.95     <.0001
```

R code

```
#means model
glm2<-glm(y ~ gt-1,
data=myostatin)
summary(glm2)
```

R output

```
      Estimate Std. Error t value Pr(>|t|)
gtc24   6.9620     0.3796  18.341 4.27e-13 ***
gtc48   5.4507     0.3796  14.359 2.67e-11 ***
gtc72   4.9112     0.3796  12.938 1.49e-10 ***
gtm24   6.0682     0.3796  15.986 4.42e-12 ***
gtm48   5.2513     0.3796  13.834 4.95e-11 ***
gtm72   3.7778     0.3796   9.952 9.62e-09 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

## *4.1.4 Time as a class variable versus time as a continuous variable*

- So far we've considered modeling time as a class variable, which allows for separate estimates at each time point. In some cases we may want to model time as a continuous variable, which imposes more constraints in the estimates.

- For example, if we allow for a straight line fit for the outcome versus time, the difference between estimates from 24 to 48 hours is necessarily the same as the difference between estimates from 48 to 72 hours. For estimates using time as a class variable, each estimate is not constrained by estimates for other time points. There may also be higher-order polynomial functions that we could use in modeling time as a continuous variable, but for now we'll just consider the straight line relationship.

- If the linearity assumption holds, there are several potential advantages to modeling time as a continuous variable despite the fact that estimates are more constrained:

  - The relationship can be expressed in a very simple, intuitive way with the slope, which expresses a change in the outcome per unit increase in x.

  - Fewer degrees of freedom are spent on the model, saving more for the error term (for the Myostatin application, time has 1 d.f. instead of (3–1)=2 with the class variable approach.

  - Estimates for values of *x* not observed can be easily obtained (e.g., at 36 hours for the Myostatin application). For application with many time points, using time as a continuous variable may be the only true alternative, since using time as a class variable in those cases may require too many d.f. for the model.

- In SAS PROC GLM, time can be modeled as a continuous variable by simply leaving the *Time* variable out of the CLASS statement. Considering the Myostatin application, *Group* and *Group*Time* and *Time* were all predictors in the model. This allowed for separate estimates for each group-time combination when *Time* was treated as a class variable. When modeling *Time* as a metric variable, separate regression lines can be obtained for each group (see next page for output). The coefficient for *Group* indicates differences between the 2 groups at the y-intercept, and the coefficient of *Group*Time* indicates differences in slopes between the 2 groups. For practice: use the output to write the regression lines for each group.

- When modeling time as a class variable, the linearity assumption can be checked informally by inspecting the PROC GPLOT graph (in previous notes) to see if the patterns look linear, or add higher order terms and see if they are significant. (We will also discuss a 'lack of fit' test for linearity, forthcoming.)

- In SAS PROC REG or the LM function in R, variables are treated as continuous variables by default and there are no CLASS statements or options. In order to model class variables, you have to create the 0/1 dummy variables yourself.

*Time as continuous for the Myostatin data*. For simplicity, we can convert hours to days. Sketch the model, matrix **X**, and vector **β** for this case. For both models, let *i* denote group, *j* denote time, *k* denote replicate. The less-than-full-rank model is given below. Note that the time variable $x_j$ is only one component of **X**.

$$Y_{ijk} = \mu + \alpha_i + \beta x_j + \gamma_i x_j + \varepsilon_{ijk}$$

where $x_j$ are the times in hours; $i$=1,2; $j$=1,2,3; $k$=1,2,3,4.

$$\boldsymbol{\beta} = \begin{pmatrix} \mu \\ \alpha_1 \\ \alpha_2 \\ \beta \\ \gamma_1 \\ \gamma_2 \end{pmatrix}$$

$$\mathbf{X} = \begin{pmatrix}
1 & 1 & 0 & 24 & 24 & 0 \\
1 & 1 & 0 & 24 & 24 & 0 \\
1 & 1 & 0 & 24 & 24 & 0 \\
1 & 1 & 0 & 24 & 24 & 0 \\
1 & 1 & 0 & 48 & 48 & 0 \\
1 & 1 & 0 & 48 & 48 & 0 \\
1 & 1 & 0 & 48 & 48 & 0 \\
1 & 1 & 0 & 48 & 48 & 0 \\
1 & 1 & 0 & 72 & 72 & 0 \\
1 & 1 & 0 & 72 & 72 & 0 \\
1 & 1 & 0 & 72 & 72 & 0 \\
1 & 1 & 0 & 72 & 72 & 0 \\
1 & 0 & 1 & 24 & 0 & 24 \\
1 & 0 & 1 & 24 & 0 & 24 \\
1 & 0 & 1 & 24 & 0 & 24 \\
1 & 0 & 1 & 24 & 0 & 24 \\
1 & 0 & 1 & 48 & 0 & 48 \\
1 & 0 & 1 & 48 & 0 & 48 \\
1 & 0 & 1 & 48 & 0 & 48 \\
1 & 0 & 1 & 48 & 0 & 48 \\
1 & 0 & 1 & 72 & 0 & 72 \\
1 & 0 & 1 & 72 & 0 & 72 \\
1 & 0 & 1 & 72 & 0 & 72 \\
1 & 0 & 1 & 72 & 0 & 72
\end{pmatrix}$$

# PROC GLM code and partial output (see the Appendix for PROC IML calc's):

```
*time as continuous variable;
proc glm data=myostatin; class group; model y = group|time / solution xpx; run;
```

```
The GLM Procedure

Dependent Variable: y
                                   Sum of
Source                     DF       Squares     Mean Square    F Value    Pr > F
Model                       3    22.20954200      7.40318067      13.11    <.0001
Error                      20    11.29140396      0.56457020
Corrected Total            23    33.50094596

R-Square     Coeff Var      Root MSE        y Mean
0.662953      13.90530      0.751379      5.403542

                                        Standard
Parameter            Estimate              Error    t Value    Pr > |t|
Intercept         7.322916667 B      0.57387509      12.76      <.0001
group      c      0.502500000 B      0.81158193       0.62      0.5428
group      m      0.000000000 B       .                .         .
time              -0.047718750 B      0.01106886      -4.31      0.0003
time*group c      0.004994792 B      0.01565373       0.32      0.7530
time*group m      0.000000000 B       .                .         .

NOTE: The X'X matrix has been found to be singular...
```

BIOS6612 review: how are the separate regression lines for the 2 groups obtained from this output?

- If $\mathbf{X}^t\mathbf{X}$ is singular, then $\tilde{\boldsymbol{\beta}}$ is not unique, but $\mathbf{X}\tilde{\boldsymbol{\beta}}$ is. Also, there may be functions of parameters in $\boldsymbol{\beta}$ that are uniquely estimable (defined soon) despite the fact that $\tilde{\boldsymbol{\beta}}$ is not unique.

- Modeling time as a class versus continuous variable is an important issue that we will discuss throughout the course.

  - Time as a class variable…
    - offers the most flexibility
    - no parametric constraints imposed across levels of time
    - uses more degrees of freedom in the model (e.g., with 4 times there are 3 d.f., 1 d.f. if you have a simple linear term for time as continuous).
    - recommended when there are relatively few times (say, five or less), for which tests for polynomial trends can still be conducted (see course notes for details).

  - Time as continuous recommended when…
    - there are many times of observation, possibly unequally spaced
    - there are different times of measurement for subjects
    - interpolating estimates and predicted values may be of interest

- Here is the fit with time as continuous using R software. Note that the 'myostatin' data is the same as presented in Section 2:

**R Code**:

```
#Model using time as continuous
contin_fit=lm(y ~ group + time + group*time,data=myostatin)
summary(contin_fit)
```

**R Output summary**:

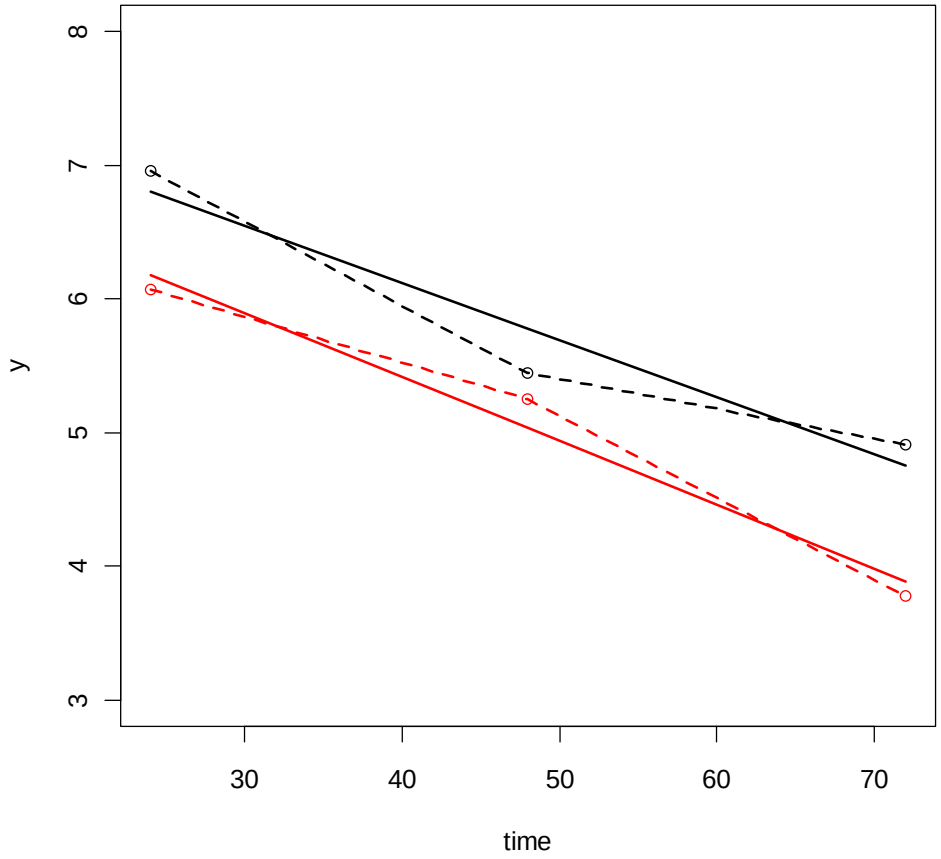
```
Call:
lm(formula = y ~ group + time + group * time, data = myostatin)

Residuals:
    Min      1Q  Median      3Q     Max
-0.8112 -0.5235 -0.1934  0.3888  1.5796

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  7.825417   0.573875  13.636 1.38e-11 ***
groupm      -0.502500   0.811582  -0.619 0.542799
time        -0.042724   0.011069  -3.860 0.000976 ***
groupm:time -0.004995   0.015654  -0.319 0.752974
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.7514 on 20 degrees of freedom
Multiple R-squared: 0.663,      Adjusted R-squared: 0.6124
F-statistic: 13.11 on 3 and 20 DF,  p-value: 5.829e-05
```

- You may notice that the signs on some of the estimates are negative, whereas with the SAS analysis they were positive. This is because in SAS, the highest level of Group (Myostatin) was set as the reference group, while with R, the lowest level of Group (Control) is set as the reference group. Make sure to remember these key differences!

- If we only include the linear term for time, then we are forcing straight-line relationships between time and y; including the interaction term allows for different slopes, while inclusion of the group term allows for different y-intercepts.

- With either approach, the interaction term is not significant. It is less significant for the time-as-continuous approach than for time-as-class. Can you see why?

*Predicted values for the Myostatin data using time as continuous (solid), and time as class (dashed, circles).*



## *4.2 Estimability of* $\boldsymbol{\beta}$

- In Section 3 we discussed beta estimates for less-than-full-rank models are not unique when $\mathbf{X}$ is not full rank, and hence care needs to be taken in interpreting results. In this section, we discuss functions of parameters that we can estimate and interpret, based on the concept of *estimability*.

- Considering the normal equations in (1), the solution to $\tilde{\boldsymbol{\beta}}$ is not unique if $\mathbf{X}$ is singular. But even when this is the case, we may find a linear function of $\tilde{\boldsymbol{\beta}}$ that does not depend on the choice of generalized inverse for $\mathbf{X}^t\mathbf{X}$. Multiply both sides of (1) on the left by an $n\times 1$ vector $\mathbf{L}$ to obtain:

$$\mathbf{L}\tilde{\boldsymbol{\beta}} = \mathbf{L}(\mathbf{X}^t\mathbf{X})^-\mathbf{X}^t\mathbf{Y}\,.$$

- If $\mathbf{L}$ can be expressed as $\mathbf{L} = \mathbf{a}^t\mathbf{X}$, then the equation becomes

$$\mathbf{L}\tilde{\boldsymbol{\beta}} = \mathbf{a}^t\underbrace{\mathbf{X}(\mathbf{X}^t\mathbf{X})^-\mathbf{X}^t}_{\boxed{\mathbf{P}_X}}\mathbf{Y}$$

- But recall that $\mathbf{X}(\mathbf{X}^t\mathbf{X})^-\mathbf{X}^t$ (the projection matrix) is invariant to the choice of generalized inverse of $\mathbf{X}^t\mathbf{X}$. In this case we say that $\mathbf{L}\boldsymbol{\beta}$ is estimable, since it has a unique (and unbiased) estimator.

- Here are some formal definitions of estimability:
  - A linear function of $\boldsymbol{\beta}$, say $\mathbf{L}\boldsymbol{\beta}$, is an estimable function if and only if there exists an unbiased estimator of $\mathbf{L}\boldsymbol{\beta}$, which is a linear function of the $Y_i$ in $\mathbf{Y}$.
  - $\mathbf{L}\boldsymbol{\beta}$ is estimable if and only if there exists a vector $\boldsymbol{a}$ such that $\mathbf{L} = \mathbf{a}^t\mathbf{X}$.

- As a consequence: if a function of $\boldsymbol{\beta}$ is not estimable, we do not have an unbiased estimator for it.

- For the less-than-full-rank model, $\tilde{\boldsymbol{\beta}} = (\mathbf{X}^t\mathbf{X})^-\mathbf{X}^t\mathbf{Y}$. We also have $\mathbf{Y} \sim N\left(\mathbf{X}\boldsymbol{\beta},\ \mathbf{I}\sigma^2\right)$.

- Using the distribution of a linear form result, letting $\mathbf{A} = (\mathbf{X}^t\mathbf{X})^-\mathbf{X}^t$, we have

$$\tilde{\boldsymbol{\beta}} = \mathbf{AY} = (\mathbf{X}^t\mathbf{X})^-\mathbf{X}^t\mathbf{Y} \sim \mathbf{N}\left[(\mathbf{X}^t\mathbf{X})^-\mathbf{X}^t\mathbf{X}\boldsymbol{\beta}, (\mathbf{X}^t\mathbf{X})^-\mathbf{X}^t\mathbf{X}(\mathbf{X}^t\mathbf{X})^-\sigma^2\right].$$

This distribution may not be unique. However, for $\mathbf{L} = \mathbf{a}^t\mathbf{X}$, we have

$$\mathbf{L} = \mathbf{L}(\mathbf{X}^t\mathbf{X})^-\mathbf{X}^t\mathbf{X},$$

and consequently

$$\mathbf{L}\tilde{\boldsymbol{\beta}} \sim \mathbf{N}\left(\mathbf{L}\boldsymbol{\beta}, \mathbf{L}(\mathbf{X}^t\mathbf{X})^-\mathbf{L}^t\sigma^2\right),$$

which is unique.

Proofs:

- If $\mathbf{L} = \mathbf{a}^t\mathbf{X}$, show that $\mathbf{L} = \mathbf{L}(\mathbf{X}^t\mathbf{X})^-\mathbf{X}^t\mathbf{X}$.

- Show that the distribution is unique.

- So how do we use these results?
  - Let $\mathbf{H} = (\mathbf{X}^t\mathbf{X})^-\mathbf{X}^t\mathbf{X}$.
  - We need to find a form of **L** so that **L**=**LH** to obtain a set of the estimable functions.
  - To accomplish this for a given experiment, first compute **H**, then express **L** generically and find the form of **LH**.
  - Finally, set **L** = **LH** and this will give us a general form that allows us to easily determine **L** that are associated with estimable functions.
- Note that **H** and $(\mathbf{X}^t\mathbf{X})^-$ are not unique. However, $\mathbf{X}(\mathbf{X}^t\mathbf{X})^-\mathbf{X}^t$ is unique, and the estimable functions of parameters that you find for a specific $(\mathbf{X}^t\mathbf{X})^-$ will be the same for any $(\mathbf{X}^t\mathbf{X})^-$ (for a one particular design).

- To illustrate, consider the Myostatin data in the one-way ANOVA model. First, compute $\mathbf{H} = (\mathbf{X}^t\mathbf{X})^-\mathbf{X}^t\mathbf{X}$. Using the generalized inverse approach discussed before (drop linearly dependent columns as you move from left to right), we obtain

$$\mathbf{H} = \left(\begin{array}{c|ccccc|c} 1/4 & -1/4 & -1/4 & -1/4 & -1/4 & -1/4 & 0 \\ \hline -1/4 & 2/4 & 1/4 & 1/4 & 1/4 & 1/4 & 0 \\ -1/4 & 1/4 & 2/4 & 1/4 & 1/4 & 1/4 & 0 \\ -1/4 & 1/4 & 1/4 & 2/4 & 1/4 & 1/4 & 0 \\ -1/4 & 1/4 & 1/4 & 1/4 & 2/4 & 1/4 & 0 \\ -1/4 & 1/4 & 1/4 & 1/4 & 1/4 & 2/4 & 0 \\ \hline 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{array}\right)\left(\begin{array}{c|cccccc|c} 24 & 4 & 4 & 4 & 4 & 4 & 4 \\ \hline 4 & 4 & 0 & 0 & 0 & 0 & 0 \\ 4 & 0 & 4 & 0 & 0 & 0 & 0 \\ 4 & 0 & 0 & 4 & 0 & 0 & 0 \\ 4 & 0 & 0 & 0 & 4 & 0 & 0 \\ 4 & 0 & 0 & 0 & 0 & 4 & 0 \\ \hline 4 & 0 & 0 & 0 & 0 & 0 & 4 \end{array}\right) = \left(\begin{array}{c|cccccc} 1 & 0 & 0 & 0 & 0 & 0 & 1 \\ \hline 0 & 1 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 1 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 1 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 1 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 \\ \hline 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{array}\right)$$

- Now for generic $\mathbf{L} = (L_1 \quad L_2 \quad L_3 \quad L_4 \quad L_5 \quad L_6 \quad L_7)$, we have

$$\mathbf{LH} = (L_1 \quad L_2 \quad L_3 \quad L_4 \quad L_5 \quad L_6 \quad L_1 - L_2 - L_3 - L_4 - L_5 - L_6)$$

- Thus, if we find coefficients such that $L_7 = L_1 - L_2 - L_3 - L_4 - L_5 - L_6$, then we have found forms of **L** that yield estimable functions. [The first 6 elements of **L** and **LH** are the same, i.e., there are no constraints.]

- Consider again the form of $\boldsymbol{\beta}$ for the one-way experiment: $\boldsymbol{\beta} = (\mu\ \kappa_1\ \kappa_2\ \kappa_3\ \kappa_4\ \kappa_5\ \kappa_6)^t$. Is $\mathbf{L}\boldsymbol{\beta} = (1\,0\,1\,0\,0\,0\,0)(\mu\ \kappa_1\ \kappa_2\ \kappa_3\ \kappa_4\ \kappa_5\ \kappa_6)^t = (\mu + \kappa_2)$ estimable?

- **L**=**LH** would require

$$(1\quad 0\quad 1\quad 0\quad 0\quad 0\quad 0) \overset{?}{=} (1\quad 0\quad 1\quad 0\quad 0\quad 0\quad 1\text{-}0\text{-}1\text{-}0\text{-}0\text{-}0)$$
$$\overset{?}{=} (1\quad 0\quad 1\quad 0\quad 0\quad 0\quad 0).$$

  Since it holds, then we have shown that indeed, $(\mu + \kappa_2)$ estimable.

- On the other hand, $\kappa_2$ is not estimable, since **L**=**LH** would require

$$(0\quad 0\quad 1\quad 0\quad 0\quad 0\quad 0) \overset{?}{=} (0\quad 0\quad 1\quad 0\quad 0\quad 0\quad 0\text{-}0\text{-}1\text{-}0\text{-}0\text{-}0)$$
$$\overset{?}{=} (0\quad 0\quad 1\quad 0\quad 0\quad 0\quad -1),$$

  which does not hold.

- For practice: is $\kappa_2 - \kappa_3$ estimable?

- Generally, are treatment differences of the form $\kappa_i - \kappa_j$ estimable?
- I mentioned that other forms of **H** would give us the same result. So let's build **H** using the MP inverse of $\mathbf{X}^t\mathbf{X}$. Using PROC IML, you will yield

$$\mathbf{H} = \frac{1}{7}\begin{pmatrix} 6 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 6 & -1 & -1 & -1 & -1 & -1 \\ 1 & -1 & 6 & -1 & -1 & -1 & -1 \\ 1 & -1 & -1 & 6 & -1 & -1 & -1 \\ 1 & -1 & -1 & -1 & 6 & -1 & -1 \\ 1 & -1 & -1 & -1 & -1 & 6 & -1 \\ 1 & -1 & -1 & -1 & -1 & -1 & 6 \end{pmatrix}$$

$$\mathbf{LH} =$$
$$1/7$$
$$(6L_1+L_2+L_3+L_4+L_5+L_6+L_7,$$
$$L_1+6L_2-L_3-L_4-L_5-L_6-L_7,$$
$$L_1-L_2+6L_3-L_4-L_5-L_6-L_7,$$
$$L_1-L_2-L_3+6L_4-L_5-L_6-L_7,$$
$$L_1-L_2-L_3-L_4+6L_5-L_6-L_7,$$
$$L_1-L_2-L_3-L_4-L_5+6L_6-L_7,$$
$$L_1-L_2-L_3-L_4-L_5-L_6+6L_7)$$

The **H** used here is $\mathbf{H} = (\mathbf{X}^t\mathbf{X})^+(\mathbf{X}^t\mathbf{X})$, where the '+' denotes the MP inverse, a special type of g-inverse.

- Although this is more cumbersome to work with than the previous form, we can show, for example, that $(\mu + \kappa_2)$ is estimable. In this case **L**=**LH** would require

Left side (**L**):

(1 0 1 0 0 0 0)

Right side (**LH**):

1/7 (6*1+0+1+0+0+0+0 1+6*0–1–0–0–0–0 1–0+6*1–0–0–0–0

1–0–1+6*0–0–0–0 1–0–1–0+6*0–0–0 1–0–1–0–0+6*1–0 1–0–1–0–0–0+6*0)

= (6/7+1/7 0 1/7+6/7 0 0 0 0)

= (1 0 1 0 0 0 0),

which indeed holds.

- SAS PROC GLM prints this general form of estimable functions with the ‘e’ option in the MODEL statement:

```
model y=group*time / E;
```

This will direct the following information to the output.

```
General Form of Estimable Functions
Effect              Coefficients
Intercept           L1
group     1         L2
group     2         L3
group     3         L4
group     4         L5
group     5         L6
group     6         L1-L2-L3-L4-L5-L6
```

This is like the first form that we derived, since SAS computes generalized inverses by dropping linearly dependent columns, moving left to right.

## *4.3 Properties of $\hat{\boldsymbol{\beta}}$, $\hat{\sigma}^2$, and linear functions of $\hat{\boldsymbol{\beta}}$*

### *4.3.1 General review of 'good' estimators: MLE, UMVU, BLU, BQU*

- Informal definitions:
  - **MLE** – maximum likelihood estimator. Values of the parameters (in terms of statistics) that maximize the likelihood function. The likelihood function is derived from the density of **Y**.
  - **UMVU** – uniformly minimum variance unbiased estimator. A statistic of **Y** that has the smallest variance in estimating a parameter within the class of unbiased estimators for that parameter. The density of **Y** must be known to derive UMVU estimators.
  - **BLU** – best linearly unbiased estimator. A statistic that has the smallest variance in estimating a parameter that is a linear function of the data **Y**. (This and BQU were developed so that 'good' estimators could be determined for the case where the pdf of **Y** is not known.)
  - **BQU** – best quadratic unbiased estimator. A statistic that has the smallest variance in estimating a parameter that is a quadratic function of the data **Y**.

## *4.3.2 Properties of estimators in the GLM*

The $\hat{\sigma}^2$ discussed below is adjusted so that it is unbiased, i.e., ($n$–$k$) used in the denominator instead of $n$.

- For Case I (*iid* normal errors), $\hat{\boldsymbol{\beta}}$ and $\hat{\sigma}^2$ are both MLE and UMVU estimators of $\boldsymbol{\beta}$ and $\sigma^2$, respectively.

- For Case II, unknown but common error distributions, $\hat{\boldsymbol{\beta}}$ is the BLU of $\boldsymbol{\beta}$ and $\hat{\sigma}^2$ is the BQU estimator of $\sigma^2$.

- When $\mathbf{X}$ does not have full rank, we use $\tilde{\boldsymbol{\beta}}$ to denote the estimator that is not unique, although it is still MLE. Since it is not unique, it is not unbiased. However, when $\mathbf{L}\boldsymbol{\beta}$ is estimable, $\mathbf{L}\tilde{\boldsymbol{\beta}}$ is the UMVU estimator (Case I) and BLU estimator (Case II) for $\mathbf{L}\boldsymbol{\beta}$.

## *4.3.3 Maximum likelihood estimators in the GLM*

- The least squares estimator $\hat{\boldsymbol{\beta}}$ is also the maximum likelihood estimator. This is shown easily by taking the partial derivative of log likelihood function with respect to $\boldsymbol{\beta}$, setting it to 0, which yields the normal equations.

- In Section 5.1, we showed that $\hat{\boldsymbol{\beta}}$ has a normal distribution with specified mean and variance, but that this distribution is not unique if the beta estimator is not unique (and hence denoted as $\tilde{\boldsymbol{\beta}}$).

- To get the MLE of $\sigma^2$, we first substitute $\hat{\boldsymbol{\beta}}$ in for $\boldsymbol{\beta}$ in the log-likelihood function, yielding a profile likelihood function that now only involves the variance parameter. The MLE is obtained by setting to 0 the partial derivative of this quantity with respect to $\sigma^2$. The solution is

$$\tilde{\sigma}^2 = (1/n)\mathbf{Y}^t(\mathbf{I} - \mathbf{P}_\mathbf{X})\mathbf{Y}.$$

- We can adjust this estimator so that it is unbiased:

$$\hat{\sigma}^2 = [1/(n-k)]\mathbf{Y}^t\left(\mathbf{I}-\mathbf{P}_\mathbf{X}\right)\mathbf{Y},$$

where $k=r(\mathbf{X})$. (If $\mathbf{X}$ has full rank, then $k=p$.)

[NOTE: The use of '~' and '^' on the variance estimator is to denote the biased and unbiased estimators, respectively, not to indicate non-unique and unique estimators, as was done for Beta.]

- Note that both variance estimators above are quadratic forms (see Section 4).

- Note that $\mathbf{I}-\mathbf{P}_\mathbf{X}$ is symmetric and invariant to choice of $(\mathbf{X}^t\mathbf{X})^-$, since $\mathbf{P}_\mathbf{X}$ also has these qualities. In addition, it is easy to show that $\mathbf{I}-\mathbf{P}_\mathbf{X}$ is idempotent:

$$\left(\mathbf{I}-\mathbf{P}_\mathbf{X}\right)\left(\mathbf{I}-\mathbf{P}_\mathbf{X}\right) = \mathbf{I}-\mathbf{P}_\mathbf{X}-\mathbf{P}_\mathbf{X}+\mathbf{P}_\mathbf{X}\mathbf{P}_\mathbf{X} = \mathbf{I}-\mathbf{P}_\mathbf{X}-\mathbf{P}_\mathbf{X}+\mathbf{P}_\mathbf{X} = \mathbf{I}-\mathbf{P}_\mathbf{X}.$$

- It follows that $\mathbf{P}_\mathbf{X}\mathbf{P}_\mathbf{X} = \mathbf{P}_\mathbf{X}$ since $\mathbf{P}_\mathbf{X}$ is in the column space of $\mathbf{X}$, as each column of $\mathbf{P}_\mathbf{X}$ is a linear combination of columns of $\mathbf{X}$. (Can you show?)

- Using the result for quadratic forms in Section 4, we can show that

$$(n-k)\hat{\sigma}^2 / \ \sigma^2 \sim \chi^2_{n-k} \ ,$$

I.e., the quantity on the left has a central chi-square distribution with *n–k* degrees of freedom.

*4.3.4 Independence of* $\hat{\boldsymbol{\beta}}$ *and* $\hat{\sigma}^2$*, see course notes.*

For Case I, this can be shown using the linear and quadratic form results from Section 4. For detail, see the lecture notes. See if you can follow the logic.

*4.3.5 BLU and BQU properties of* $\hat{\boldsymbol{\beta}}$ *and* $\hat{\sigma}^2$

$\mathbf{L}\hat{\boldsymbol{\beta}}$ is BLU and $\hat{\sigma}^2$ is BQU (General error distribution case.) The Gauss-Markov Theorem states that $\mathbf{L}\hat{\boldsymbol{\beta}}$ is the BLU estimator of $\mathbf{L}\boldsymbol{\beta}$. Showing $\hat{\sigma}^2$ is the BQU estimator of $\sigma^2$ involves tedious algebra.

## *4.4 Standard errors and confidence intervals*

- Let **C** be a matrix with rows $\mathbf{c}_i^t$, $i$=1,…,$q$. We typically denote one $\mathbf{c}_i^t$ as **L**. Concerning the functions of parameters $\boldsymbol{\theta} = \mathbf{C}\boldsymbol{\beta} - \mathbf{h}$, we may be interested in developing confidence intervals. There are two basic types:

- <u>One-at-a-time confidence intervals</u>. This means that each $\theta_i$ is treated individually, and a (1–$\alpha$) CI is determined separately for each $\theta_i = \mathbf{c}_i^t\boldsymbol{\beta}$ (where $\mathbf{c}_i^t$ is the $i^{\text{th}}$ row of **C**):

$$\mathbf{c}_i^t\hat{\boldsymbol{\beta}} \mp t_{a/2,n-k}\sqrt{\hat{Var}[\mathbf{c}_i^t\hat{\boldsymbol{\beta}}]}, \qquad i=1,\ldots,q$$

- <u>Simultaneous confidence intervals</u>. This means that all of the $\theta_i$ are treated simultaneously, and CIs are determined for each $\theta_i$ such that the probability is equal to 1–$\alpha$ that the $q$ intervals simultaneously cover their respective $\theta_i$.

- Standard errors of Beta estimates: For $\mathbf{L\beta}$ that is estimable, previous theoretical results imply that $SE(\mathbf{L}\hat{\boldsymbol{\beta}}) = \sqrt{\mathbf{L}(\mathbf{X}^t\mathbf{X})^{-}\mathbf{L}^t\sigma^2}$. Since $\sigma^2$ is typically unknown, the quantity is usually estimated with $\hat{SE}(\mathbf{L}\hat{\boldsymbol{\beta}}) = \sqrt{s^2\mathbf{L}(\mathbf{X}^t\mathbf{X})^{-}\mathbf{L}^t}$, where $s^2 = \left[\mathbf{Y}^t(\mathbf{I}-\mathbf{P}_X)\mathbf{Y}\right] / (n-k)$, the MSE.

  - Practice 1: use PROC IML to replicate SE's for the Myostatin application and compare with output from PROC GLM.

  - Practice 2: (1) Compute (a) the point estimate, (b) standard error, and (c ) confidence interval for $\mu+\kappa_1$ for the Myostatin data in the one-way model. (2) Repeat these steps for $\kappa_1-\kappa_2$. Note that for both of these functions of parameters, $\mathbf{L\beta}$ is estimable.

Note that for $\kappa_1-\kappa_2$, L=(0 1 -1 0 0 0 0). This compares effects of 24 and 48 hours for the Control group.

Note that *s* = Root MSE = 0.759. Also note that $\mathbf{L}(\mathbf{X}^t\mathbf{X})^{-}\mathbf{L}^t$ is a scalar. See notes for overview of calculations.