Chapter 2
Basic Multilevel Models

Birth Order Data Analysis

For this demonstration we will again use the `birth.sas7bdat` data file (after deleting the outlier as shown in Chapter 1). Accompanying SAS code is in the `Birth_RE.sas` file.

Random Effects ANOVA

We can get a crude sense of how meaningful between-family differences in Math IQ are by computing and plotting the distribution of family mean IQ. We do this by first outputting the mean IQ for each family using PROC MEANS. We will use these means in two plots, an index plot and a histogram of family means. For the index plot, we merge the means back into the original data (producing the data set called `plot`).

```
proc sort data=birth; by mom_id; run;
proc means data=birth noprint; by mom_id;
 var math;
 output out=mean mean=mathm;
run;

proc print data=mean(obs=10); run;
data mean; set mean; index = _N_; run;
data plot; merge birth mean; by mom_id;
run;
proc print data=plot (obs=20);
 var mom_id index math _freq_ mathm;
run;
```

This is some of the data produced by PROC MEANS, now in the `means` data set:

```
           Obs          mom_id     _TYPE_     _FREQ_          mathm

             1               3        0          1             102
             2               4        0          1             105
             3               8        0          2            99.5
             4              20        0          1             112
             5              25        0          1             105
             6              43        0          2              81
             7              44        0          1             100
             8              49        0          2           107.5
             9              50        0          2           109.5
            10              57        0          1             135
```

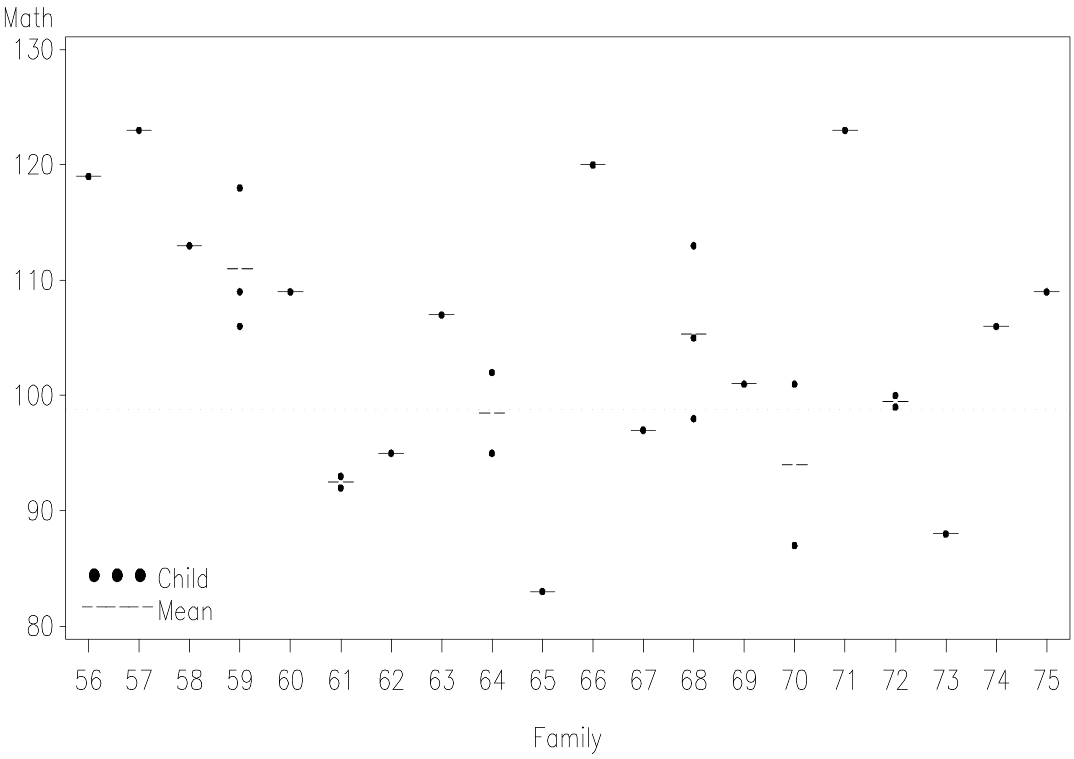
And these means are merged back into the original data in the `plot` data set:

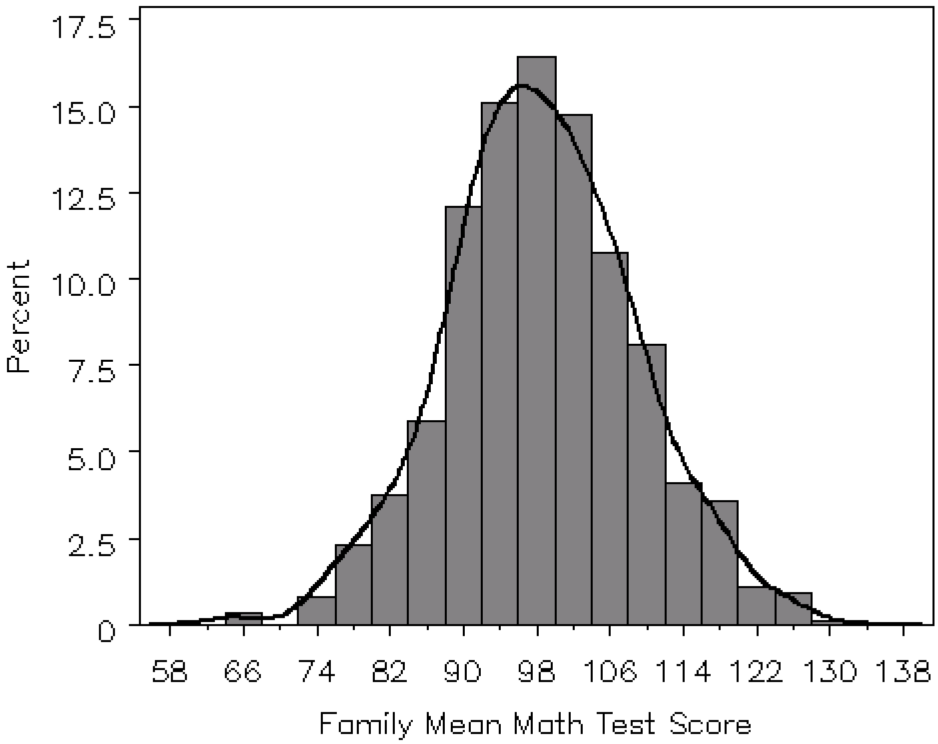
```
   Obs          mom_id     index           math     _FREQ_           mathm

     1               3       1              102        1               102
     2               4       2              105        1               105
     3               8       3              102        2              99.5
     4               8       3               97        2              99.5
     5              20       4              112        1               112
     6              25       5              105        1               105
     7              43       6               97        2                81
     8              43       6               65        2                81
     9              44       7              100        1               100
    10              49       8              126        2             107.5
```

```
    11              49       8             89       2           107.5
    12              50       9            111       2           109.5
    13              50       9            108       2           109.5
    14              57      10            135       1             135
```

Next, we produce our plots. Note that the index plot is made on a subset of the families in the data (the 56th through 75th families) and that we selected only families with 2+ children in the data for the histogram because otherwise the family mean and child score are identical.

```
goptions vsize=5 hsize=8 htext=1.25;
symbol1 value=dot h=.5 w=.5 color=black repeat=1;
symbol2 font=simplex value="--" color=black repeat=1;
axis1 minor=none label=("Math");
axis2 minor=none offset=(2) label=("Family") order=(56 to 75 by 1);
legend1 position=(inside bottom left) offset=(2) down=2 label=none
value=('Child' 'Mean');
proc gplot data=plot; where 55<index<=75;
 plot (math mathm)*index/legend=legend1 overlay vref=98.8 lvref=33
haxis=axis2 vaxis=axis1;
run;quit;

goptions hsize=5 vsize=4;
proc univariate data=mean; where _FREQ_>1;
 var mathm;
 histogram/ cfill=red kernel(color=black w=2) haxis=axis1;
 axis1 label=("Family Mean Math Test Score");
run;
```





The index plot shows that there is both within- and between-family variation. There are individual differences among siblings within a family, but also differences in mean math IQ across families. Focusing on the across-family differences, the histogram shows a great deal of family-to-family variation in math IQ, and that this variation follows the form of a normal distribution. Of course, some of the variation is due to simple sampling error (since each family mean is computed from very few observations), so we would like to more formally evaluate within- versus between-family IQ differences. The random-effects ANOVA model provides a way to decompose this variance.

The RE-ANOVA model is

Level 1:

$$y_{ij} = \beta_{0j} + r_{ij} \qquad r_{ij} \sim N\left(0, \sigma^2\right)$$

Level 2:

$$\beta_{0j} = \gamma_{00} + u_{0j} \qquad u_{0j} \sim N\left(0, \tau_{00}\right)$$

Reduced-Form:

$$y_{ij} = \gamma_{00} + u_{0j} + r_{ij}$$

The code for fitting this model is shown here:

```
proc mixed data=demo.birth method=reml covtest cl noclprint;
  class mom_id;
  model math = / solution ddfm=bw;
  random intercept / subject=mom_id;
run;
```

Syntax Notes:

- PROC MIXED statement: invokes the MIXED procedure
    - METHOD=REML tells MIXED to use the REstricted Maximum Likelihood Estimator to fit the model.
    - COVTEST produces z-tests for the variance parameters of the model.
    - CL produces confidence limits for the variance parameters of the model.
    - NOCLPRINT suppresses printing of all levels of variables declared in the CLASS statement.
- CLASS statement: Used to declare classification (nominal) variables used in the model specification.
    - We have listed the ID variable for the nesting (`mom_id`). It is not essential to include this here, but it can help to avoid errors (see description of RANDOM statement).
- MODEL statement: Used to define the outcome variable (left of =) and predictors with fixed effects in the model (right of =). MIXED will estimate a fixed effect for any predictor included in this statement. MIXED will estimate *G*-1 effects for any *G*-level categorical predictor defined previously in the CLASS statement. A fixed intercept is estimated by default.
    - SOLUTION tells SAS to report the table of fixed effect estimates, standard errors, and p-values.
    - DDFM tells SAS which method to use for computing degrees of freedom for testing fixed effects. BW is the heuristic “Between and Within” method which draws an analogy to repeated measures ANOVA for computing degrees of freedom.
- RANDOM statement: Used to define the random effects of the model. A random intercept is *not* included by default and must be defined by putting the keyword “Intercept.”
    - SUBJECT is used to indicate the nesting within the data (here, siblings within `mom_ID`). *If this ID variable is numeric and the data is sorted on this variable in advance of the MIXED procedure then it is not necessary that it be declared in the CLASS statement. But, if it is not sorted, results will be wrong.*

Results include some descriptive information:

```
                             Model Information

          Data Set                     WORK.BIRTH
          Dependent Variable           math
          Covariance Structure         Variance Components
          Subject Effect               mom_id
          Estimation Method            REML
          Residual Variance Method     Profile
          Fixed Effects SE Method      Model-Based
          Degrees of Freedom Method    Between-Within


                                 Dimensions

                    Covariance Parameters             2
                    Columns in X                      1
                    Columns in Z Per Subject          1
                    Subjects                       2207
                    Max Obs Per Subject               4


                           Number of Observations

                Number of Observations Read            3312
                Number of Observations Used            3312
                Number of Observations Not Used           0
```

Note that in the “Dimensions” table, SAS reports 2 (co)variance parameters, corresponding to σ^2 and τ_{00}. “Columns in X” refers to number of columns in the design matrix for the fixed effects (here just a single column of ones for the fixed intercept parameter), whereas “Columns in Z” refers to the number of columns in the design matrix for the random effects (also a single column of ones in this case, for the random intercept). “Subjects” reports the number of unique units declared in the SUBJECT option of the RANDOM statement (here, 2207 families).

The next bit of output tells us that the model converged. *Do not interpret your model estimates if the model fails to converge*.

```
                              Iteration History

        Iteration    Evaluations    -2 Res Log Like       Criterion

                0              1     26107.69744119
                1              3     25942.89945587      0.00000931
                2              1     25942.80545103      0.00000001


                         Convergence criteria met.
```

We are next provided with information about the variance parameters of the model:

```
                      Covariance Parameter Estimates

                               Standard       Z
Cov Parm    Subject  Estimate     Error   Value    Pr > Z   Alpha     Lower

Intercept   mom_id    52.1217    4.3981   11.85    <.0001    0.05   44.4716
Residual               102.30    4.0334   25.36    <.0001    0.05   94.8366

                           Covariance Parameter
                                 Estimates

                       Cov Parm    Subject     Upper

                       Intercept   mom_id    61.9420
                       Residual               110.69
```

The “Covariance Parameter Estimates” table provides estimates for the random intercept variance $\hat{\tau}_{00} = 52.12$ and the residual variance $\hat{\sigma}^2 = 102.30$. The corresponding ICC is $52.12/\left(52.12+102.30\right) = .34$. The standard errors, z-values and p-values shown here for the variance estimates were obtained from the COVTEST option of the MIXED statement, whereas the lower and upper confidence limits for the 95% confidence intervals were obtained from the CL option of the MIXED procedure. *Note that the z-tests and confidence intervals are computed using different assumptions about the sampling distributions of the variance parameters; hence it is possible to find p-values for the z-test greater than .05 simultaneously with CIs that do not include zero.*

SAS also provides some fit statistics:

```
                              Fit Statistics

                   -2 Res Log Likelihood         25942.8
                   AIC (smaller is better)       25946.8
                   AICC (smaller is better)      25946.8
                   BIC (smaller is better)       25958.2
```

We defer discussion of these until later.

Finally, we obtain the estimates for the fixed effects:

```
                        Solution for Fixed Effects

                              Standard
     Effect       Estimate       Error      DF    t Value    Pr > |t|

     Intercept     98.9115      0.2386    2206     414.50      <.0001
```

This table shows that the average value of Math IQ is 98.91. Note that this will not exactly equal the grand mean ignoring clustering because MIXED implicitly adjusts for the partial redundancy in data due to dependence. This table is produced only when the SOLUTION option is included on the MODEL statement.

Adding Lower-Level Predictors to the Model

We will now add fixed effects for birth order, cohort and birth order × cohort to the model at Level 1. Our model will now be:

Level 1:

$$\begin{aligned} Math_{ij} = \beta_{0j} + \beta_{1j}Second_{ij} + \beta_{2j}Third_{ij} + \beta_{3j}Fourth_{ij} + \beta_{4j}Old_{ij} + \\ \beta_{5j}Second_{ij} \times Old_{ij} + \beta_{6j}Third_{ij} \times Old_{ij} + \beta_{7j}Fourth_{ij} \times Old_{ij} + r_{ij} \end{aligned}$$

$$r_{ij} \sim N\left(0, \sigma^2\right)$$

Level 2:

$$\beta_{0j} = \gamma_{00} + u_{0j} \qquad u_{0j} \sim N\left(0, \tau_{00}\right)$$

$\beta_{pj} = \gamma_{p0}$ for $p > 0$

Reduced-Form:

$$\begin{aligned} Math_{ij} = \gamma_{00} + \gamma_{10}Second_{ij} + \gamma_{20}Third_{ij} + \gamma_{30}Fourth_{ij} + \gamma_{40}Old_{ij} + \\ \gamma_{50}Second_{ij} \times Old_{ij} + \gamma_{60}Third_{ij} \times Old_{ij} + \gamma_{70}Fourth_{ij} \times Old_{ij} + \\ u_{0j} + r_{ij} \end{aligned}$$

To fit the model in PROC MIXED, we first sort the data in descending order by `brthordr` and `cohort` so that we can make the reference categories first born children and children in the younger cohort using the ORDER=DATA option.

```
proc sort data=birth; by descending brthordr descending cohort; run;
proc mixed data=birth method=reml covtest cl order=data noclprint;
  class brthordr cohort mom_id;
  model math = brthordr cohort cohort*brthordr / solution ddfm=bw;
  random intercept / subject=mom_id;
run;
```

`Brthordr`, `cohort`, and `mom_id` are both declared in the CLASS statement and then `brthordr`, `cohort`, and `cohort*brthordr` fixed effects are included by adding these predictors to the MODEL statement. Abridged results follow:

```
                              Dimensions

                    Covariance Parameters            2
                    Columns in X                    15
                    Columns in Z per Subject         1
                    Subjects                      2207
                    Max Obs per Subject              4
```

In the “Dimensions” table, SAS reports 15 columns in X. These are 1 (intercept) + 4 (birth orders) + 2 (cohorts) + 4×2 (birth orders x cohorts). All levels of the CLASS variables are included in the count despite the fact that estimates are *not* produced for all of them (e.g., 3 dummy variables suffice to summarize the 4 levels of birth order).

```
                            Covariance Parameter Estimates

                                Standard       Z
Cov Parm    Subject  Estimate      Error   Value     Pr > Z   Alpha     Lower     Upper

Intercept   mom_id    49.2755     4.2992   11.46     <.0001    0.05   41.8264   58.9196
Residual               101.61     4.0050   25.37     <.0001    0.05   94.1991    109.94


                              Solution for Fixed Effects

                                                     Standard
 Effect           brthordr      cohort  Estimate        Error    DF  t Value  Pr > |t|

 Intercept                                101.50       0.4742  2206   214.02    <.0001
 brthordr                4                -3.4931      1.2264  1098    -2.85    0.0045
 brthordr                3                -2.7351      0.8037  1098    -3.40    0.0007
 brthordr                2                -1.5302      0.6672  1098    -2.29    0.0220
 brthordr                1                      0           .     .        .         .
 cohort                     1             -3.2685      0.6072   460    -5.38    <.0001
 cohort                     0                   0           .     .        .         .
 brthordr*cohort         4  1            -0.08082      2.0917  1098    -0.04    0.9692
 brthordr*cohort         4  0                   0           .     .        .         .
 brthordr*cohort         3  1             -0.9942      1.2808  1098    -0.78    0.4378
 brthordr*cohort         3  0                   0           .     .        .         .
 brthordr*cohort         2  1              0.3419      0.9261  1098     0.37    0.7120
 brthordr*cohort         2  0                   0           .     .        .         .
 brthordr*cohort         1  1                   0           .     .        .         .
 brthordr*cohort         1  0                   0           .     .        .         .


                            Type 3 Tests of Fixed Effects

                                   Num      Den
               Effect               DF       DF     F Value    Pr > F

               brthordr              3     1098       11.53    <.0001
               cohort                1      460       30.02    <.0001
               brthordr*cohort       3     1098        0.34    0.7961
```

The conditional (residual) variance estimates seen in the “Covariance Parameter Estimates” table can be compared to the marginal (total) variance estimates obtained from the random-effects ANOVA results to see variance explained at each level of the model. Note that it is the between-family variance that has been most reduced by the inclusion of the Level 1 predictors.

The “Type 3 Tests of Fixed Effects” table gives omnibus (multiple degrees of freedom) tests of the main effects and interaction in the model. The “Solution for Fixed Effects” table shows the individual effects of the coding variables. Within the later table, the reference categories for CLASS variables are indicated with estimates of zero and dots for significance tests.

Adding Upper-Level Predictors to the Model

We now include the mother's age when she had her first child as a Level 2 predictor of intercepts, to undo confounding between maternal age and birth order:

Level 1:

$$\begin{aligned} Math_{ij} = {} & \beta_{0j} + \beta_{1j}Second_{ij} + \beta_{2j}Third_{ij} + \beta_{3j}Fourth_{ij} + \beta_{4j}Old_{ij} + \\ & \beta_{5j}Second_{ij} \times Old_{ij} + \beta_{6j}Third_{ij} \times Old_{ij} + \beta_{7j}Fourth_{ij} \times Old_{ij} + r_{ij} \end{aligned}$$

$$r_{ij} \sim N\left(0, \sigma^2\right)$$

Level 2:

$$\beta_{0j} = \gamma_{00} + \gamma_{01}Birthage_j + u_{0j} \qquad u_{0j} \sim N\left(0, \tau_{00}\right)$$

$\beta_{pj} = \gamma_{p0}$ for $p > 0$

Reduced-Form:

$$\begin{aligned} Math_{ij} = {} & \gamma_{00} + \gamma_{01}BirthAge_j + \gamma_{10}Second_{ij} + \gamma_{20}Third_{ij} + \gamma_{30}Fourth_{ij} + \\ & \gamma_{40}Old_{ij} + \gamma_{50}Second_{ij} \times Old_{ij} + \gamma_{60}Third_{ij} \times Old_{ij} + \gamma_{70}Fourth_{ij} \times Old_{ij} + \\ & u_{0j} + r_{ij} \end{aligned}$$

The corresponding MIXED syntax follows. Note that **`brthage`** is first mean-centered to simplify interpretation of the model intercept.

```
proc standard data=birth out=birthcent m=0;
 var brthage;
run;

proc mixed data=birthcent method=reml covtest cl order=data noclprint;
  class brthordr cohort mom_id;
  model math = brthordr cohort cohort*brthordr brthage /
               solution ddfm=bw;
  random intercept / subject=mom_id;
run;
```

Abridged results follow:

```
                           Dimensions

             Covariance Parameters            2
             Columns in X                    16
             Columns in Z Per Subject         1
             Subjects                      2207
             Max Obs Per Subject              4
```

```
                            Covariance Parameter Estimates

                                Standard       Z
Cov Parm    Subject  Estimate      Error   Value    Pr > Z   Alpha     Lower     Upper

Intercept   mom_id    43.3318     4.1352   10.48    <.0001    0.05   36.2454   52.7324
Residual               101.65     3.9923   25.46    <.0001    0.05   94.2625    109.96


                              Solution for Fixed Effects

                                                     Standard
 Effect           brthordr      cohort  Estimate        Error    DF  t Value  Pr > |t|

 Intercept                               99.1133       0.5175  2205   191.51    <.0001
 brthordr                4                0.1172       1.2627  1098     0.09    0.9261
 brthordr                3                0.1692       0.8397  1098     0.20    0.8403
 brthordr                2               0.01720       0.6746  1098     0.03    0.9797
 brthordr                1                     0            .     .        .         .
 cohort                            1     -0.2755       0.6604   460    -0.42    0.6768
 cohort                            0           0            .     .        .         .
 brthordr*cohort         4  1            -2.4697       2.0825  1098    -1.19    0.2359
 brthordr*cohort         4  0                  0            .     .        .         .
 brthordr*cohort         3  1            -2.7984       1.2775  1098    -2.19    0.0287
 brthordr*cohort         3  0                  0            .     .        .         .
 brthordr*cohort         2  1            -0.5900       0.9186  1098    -0.64    0.5208
 brthordr*cohort         2  0                  0            .     .        .         .
 brthordr*cohort         1  1                  0            .     .        .         .
 brthordr*cohort         1  0                  0            .     .        .         .
 brthage                                  0.7325      0.06889  2205    10.63    <.0001


                            Type 3 Tests of Fixed Effects

                                    Num      Den
               Effect                DF       DF    F Value    Pr > F

               brthordr               3     1098       1.35    0.2557
               cohort                 1      460       7.30    0.0071
               brthordr*cohort        3     1098       1.84    0.1385
               brthage                1     2205     113.07    <.0001
```

Note the increment to “Columns in X” associated with the inclusion of **`brthage`**. The omnibus test for **`brthordr`** in the “Type 3 Tests of Fixed Effects” table is now non-significant. Similarly, in the “Solution for Fixed Effects” table, the effect of each dummy variable for birth order is non-significant.

One dummy variable for the birth order by cohort interaction is significant, suggesting a significant difference between first and third born children in the older cohort, but this should be interpreted cautiously (or not at all) given the lack of a significant omnibus cohort by birth order interaction.

Beta Interferon Analysis

For this demonstration we use the **`beta.sas7bdat`** data file. The data has been provided courtesy of Dr. Gideon Koren, who runs the Motherisk Program at the University of Toronto, Hospital for Sick Children. Accompanying SAS code is in **`betaint.sas`**. Variables are:

`MID`	A unique numeric identifier for each mother.
`Group`	A nominal variable indicating which group the mother belongs to, Multiple Sclerosis – Took Beta-Interferon (B-Ifn), Multiple Sclerosis – Discontinued Medication (Disc), or Healthy Control (HCntrl).
`GestAge`	Gestational age of the infant at delivery, in weeks.
`MGain_lb`	Mother's weight gain during pregnancy, in pounds.
`CWeight_lb`	Infant's birth weight, in pounds.

Exploratory Data Analysis

We will begin by examining the characteristics of the data. The raw data is shown below:

```
proc print data=demo.beta;
run;
```

```
                                   Gest                CWeight_
           Obs    MID    group      Age    MGain_lb       lb

             1      1    Disc       38         20       7.76367
             2      1    Disc       38         20       7.54453
             3      1    Disc       38         20       7.79497
             4      7    B-Ifn      38         24       7.63845
             5      8    B-Ifn      40         32       6.44885
             6      9    B-Ifn      32         21       5.35317
             7     10    B-Ifn      38         24       7.51323
             8     10    B-Ifn      38         24       6.57407
             9     11    B-Ifn      38         30       7.32540
            10     12    B-Ifn      40         45       7.76367
            11     13    B-Ifn      38         30       8.20194
            12     14    B-Ifn      40         30       5.69753
            13     15    B-Ifn      38        -15       8.20194
            14     16    B-Ifn      35         43       6.63668
            15     17    Disc       38         41       8.17063
            16     18    Disc       38         19       7.51323
            17     18    Disc       37         25       8.38977
            18     18    Disc       38          9       9.14109
            19     19    Disc       38         33       8.32716
            20     20    Disc       30         23       5.22795
            21     21    Disc       38          .       6.38624
            22     22    Disc       38         30       7.51323
            23     24    Disc       41          .       8.20194
            24     25    Disc       38         76       9.20370
            25     25    Disc       38         45       8.13933
            26     25    Disc       39         55       8.64021
            27     26    Disc       35         18       6.57407
            28     26    Disc       35         24       6.57407
```

```
             29      28     HCntrl     38          9       8.76543
             30      29     HCntrl     39         40       8.70282
             31      30     HCntrl     38         60       8.13933
             32      31     HCntrl     39         50       8.32716
             33      32     HCntrl     39         23       8.13933
             34      33     HCntrl     35         75       8.51499
             35      34     HCntrl     38         43       8.45238
             36      35     HCntrl     39         24       8.20194
             37      36     HCntrl     37         22       6.68677
             38      37     HCntrl     37         40       6.10450
             39      38     HCntrl     39         75       9.14109
             40      39     HCntrl     39         25       8.89065
             41      40     HCntrl     39         25       7.57584
             42      41     HCntrl     38         31       7.76367
             43      43     HCntrl     38        118       9.01587
             44      43     HCntrl     38         86       9.39153
             45      44     HCntrl     39         28       9.89242
             46      45     HCntrl     39         35       8.45238
```

Note that there is some missing data on the maternal weight gain covariate.

The extent of nesting within the data is shown here:

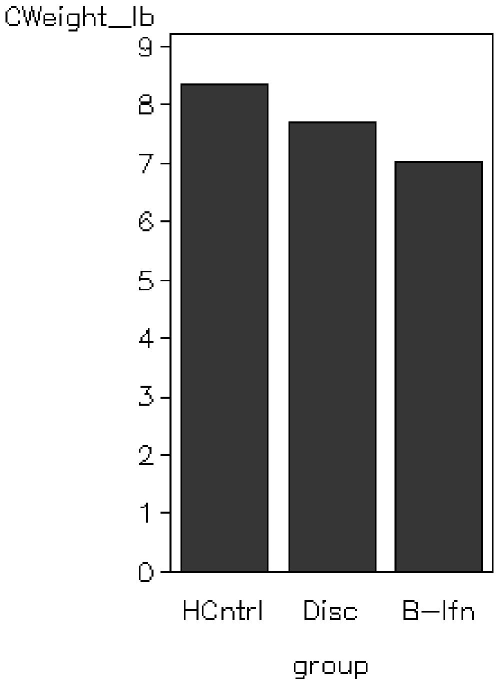
```
proc freq data=demo.beta noprint;
  table MID /out=MIDfreq;
run;
proc freq data=MIDfreq;
  table count;
run;
```

```
                         The FREQ Procedure

                           Frequency Count

                                            Cumulative    Cumulative
     COUNT     Frequency      Percent        Frequency      Percent
     ---------------------------------------------------------------

         1          31        83.78              31          83.78
         2           3         8.11              34          91.89
         3           3         8.11              37         100.00
```

The mean birth weights for the three groups are computed and graphed here:

```
proc means data=demo.beta N mean median min max;
 class group;
 var gestage mgain_lb cweight_lb;
run;

proc gchart data=demo.beta;
 vbar group /discrete type=mean sumvar=cweight_lb axis=axis1
            descending;
 pattern value=solid color=red;
 axis1 label=("CWeight_lb") minor=none;
run; quit;
```

```
group     Obs    Variable       N           Mean         Median        Minimum
──────────────────────────────────────────────────────────────────────────────

B-Ifn      11    GestAge       11     37.7272727     38.0000000     32.0000000
                 MGain_lb      11     26.1818182     30.0000000    -15.0000000
                 CWeight_lb    11      7.0322671      7.3253968      5.3531746

Disc       17    GestAge       17     37.3529412     38.0000000     30.0000000
                 MGain_lb      15     30.5333333     24.0000000      9.0000000
                 CWeight_lb    17      7.7121071      7.7949735      5.2279541

HCntrl     18    GestAge       18     38.2222222     38.5000000     35.0000000
                 MGain_lb      18     44.9444444     37.5000000      9.0000000
                 CWeight_lb    18      8.3421174      8.4523810      6.1044974
──────────────────────────────────────────────────────────────────────────────


                            N
                  group    Obs    Variable           Maximum
                  ────────────────────────────────────────────

                  B-Ifn     11    GestAge         40.0000000
                                  MGain_lb        45.0000000
                                  CWeight_lb       8.2019400

                  Disc      17    GestAge         41.0000000
                                  MGain_lb        76.0000000
                                  CWeight_lb       9.2037037

                  HCntrl    18    GestAge         39.0000000
                                  MGain_lb       118.0000000
                                  CWeight_lb       9.8924162
                  ────────────────────────────────────────────
```



Random-Effects ANOVA Model

Fitting a random-effects ANOVA model will allow us to determine the intraclass correlation, and to partition the variance in birth weights into within- and between-mother components.

Level 1:

$$CWeight\_lb_{ij} = \beta_{0j} + r_{ij} \qquad r_{ij} \sim N\left(0, \sigma^2\right)$$

Level 2:

$$\beta_{0j} = \gamma_{00} + u_{0j} \qquad u_{0j} \sim N\left(0, \tau_{00}\right)$$

Reduced-Form:

$$CWeight\_lb_{ij} = \gamma_{00} + u_{0j} + r_{ij}$$

The corresponding SAS code is

```
proc mixed data=demo.beta method=reml noclprint;
  class MID;
  model CWeight_lb =  / solution alpha=.05 ddfm=kr;
  random intercept / subject=MID vcorr;
run;
```

Note that no inference tests were requested on the variance components given the very few mothers with multiple siblings that provide information on this partitioning. In addition, the DDFM=KR option in the MODEL statement invokes the Kenward-Rogers method for estimating standard errors and degrees of freedom for the fixed effects of the model. Standard errors are adjusted for small-sample bias in the variance parameter estimates and degrees of freedom are approximated via the Satterthwaite procedure. This method of testing fixed effects is optimal for small samples.

Output:

```
                         Model Information

         Data Set                     DEMO.BETA
         Dependent Variable           CWeight_lb
         Covariance Structure         Variance Components
         Subject Effect               MID
         Estimation Method            REML
         Residual Variance Method     Profile
         Fixed Effects SE Method      Kenward-Roger
         Degrees of Freedom Method    Kenward-Roger


                            Dimensions

                  Covariance Parameters            2
                  Columns in X                     1
                  Columns in Z Per Subject         1
                  Subjects                        37
                  Max Obs Per Subject              3
```

```
                           Number of Observations

                 Number of Observations Read              46
                 Number of Observations Used              46
                 Number of Observations Not Used           0

                              Iteration History

         Iteration    Evaluations    -2 Res Log Like       Criterion

                 0              1       138.41377055
                 1              2       132.32910598      0.01819786
                 2              1       131.78710783      0.00278792
                 3              1       131.71216977      0.00007216
                 4              1       131.71037070      0.00000005
                 5              1       131.71036943      0.00000000

                         Convergence criteria met.

                  Estimated V Correlation Matrix for MID 1

                   Row        Col1        Col2        Col3

                     1      1.0000      0.7733      0.7733
                     2      0.7733      1.0000      0.7733
                     3      0.7733      0.7733      1.0000

                       Covariance Parameter Estimates

                      Cov Parm      Subject    Estimate

                      Intercept     MID          0.9488
                      Residual                   0.2781

                               Fit Statistics

                    -2 Res Log Likelihood           131.7
                    AIC (smaller is better)         135.7
                    AICC (smaller is better)        136.0
                    BIC (smaller is better)         138.9

                         Solution for Fixed Effects

                           Standard
Effect        Estimate        Error      DF    t Value    Pr > |t|     Alpha

Intercept       7.7456       0.1801    35.4      43.01      <.0001      0.05

                         Solution for Fixed Effects

                     Effect           Lower       Upper

                     Intercept       7.3802      8.1110
```

The ICC is shown by the VCORR output to be .7733. The between-mothers variance is .9488 and the within-mother variance is .2781. The pooled average for birth weight is 7.7456 lbs.

Adding Upper-Level Predictors to the Model

We now examine whether the mean differences in birth weight that we observed previously are statistically significant when accounting for dependence within mothers.

Level 1:

$$CWeight\_lb_{ij} = \beta_{0j} + r_{ij} \qquad r_{ij} \sim N\left(0, \sigma^2\right)$$

Level 2:

$$\beta_{0j} = \gamma_{00} + \gamma_{01} B\text{-}Ifn + \gamma_{02} Disc + u_{0j} \qquad u_{0j} \sim N\left(0, \tau_{00}\right)$$

Reduced-Form:

$$CWeight\_lb_{ij} = \gamma_{00} + \gamma_{01} B\text{-}Ifn + \gamma_{02} Disc + u_{0j} + r_{ij}$$

The corresponding SAS code is:

```
proc mixed data=demo.beta method=reml noclprint;
  class MID Group;
  model CWeight_lb = Group / solution alpha=.05 ddfm=kr;
  random intercept / subject=MID vcorr;
  estimate "b-ifn v. disc" group 1 -1 0 / e cl;
run;
```

Syntax Notes:

- The ESTIMATE statement: Used to conduct null hypothesis tests on linear combinations of parameter estimates. Given the way the model has been specified, we will obtain tests of the B-Ifn mean versus HCntrl and the Disc mean versus HCntrl, but we have no test of B-Ifn versus Disc. To contrast the B-Ifn group from the Disc group, we need to test null hypothesis: $H_0 : \gamma_{01} - \gamma_{02} = 0$. This follows from the fact that

$$\mu_{B\text{-}Ifn} = \gamma_{00} + \gamma_{01}$$
$$\mu_{Disc} = \gamma_{00} + \gamma_{02}$$

  It then follows that $\mu_{B\text{-}Ifn} - \mu_{Disc} = \left(\gamma_{00} + \gamma_{01}\right) - \left(\gamma_{00} + \gamma_{02}\right) = \gamma_{01} - \gamma_{02}$.
    - You designate the label for the test in quotes, then indicate which effects you are combining using the name of the predictor, and you provide weights for the effect. Here, the weights are 1, -1, and 0 for the three levels of the group variable.
    - The E option asks MIXED to report the weights used to compute the linear combination (useful for checking that you specified the linear combination correctly).
    - The CL option requests a 95% confidence interval for the linear combination.

Abridged output is shown here:

```
                  Estimated V Correlation Matrix for MID 1

                   Row        Col1        Col2        Col3

                     1      1.0000      0.7198      0.7198
                     2      0.7198      1.0000      0.7198
                     3      0.7198      0.7198      1.0000


                       Covariance Parameter Estimates

                      Cov Parm      Subject    Estimate

                      Intercept     MID          0.7107
                      Residual                   0.2766



                         Solution for Fixed Effects

                                 Standard
   Effect      group   Estimate     Error    DF  t Value  Pr > |t|   Alpha

   Intercept             8.3001    0.2399  35.1    34.59    <.0001    0.05
   group       B-Ifn    -1.2688    0.3935  34.8    -3.22    0.0027    0.05
   group       Disc     -0.7570    0.3862  31.6    -1.96    0.0588    0.05
   group       HCntrl         0         .     .        .         .       .

                         Solution for Fixed Effects

                  Effect      group      Lower       Upper

                  Intercept             7.8130      8.7872
                  group       B-Ifn    -2.0678     -0.4697
                  group       Disc     -1.5439     0.03002
                  group       HCntrl         .           .


                       Type 3 Tests of Fixed Effects

                             Num     Den
               Effect         DF      DF    F Value    Pr > F

               group           2    32.8       5.52    0.0086


                       Coefficients for b-ifn v. disc

                       Effect        group       Row1

                       Intercept
                       group         B-Ifn          1
                       group         Disc          -1
                       group         HCntrl
```

```
                                Estimates

                            Standard
 Label           Estimate      Error     DF   t Value   Pr > |t|    Alpha

 b-ifn v. disc    -0.5118     0.4346   32.1     -1.18     0.2475     0.05

                                Estimates

                  Label              Lower       Upper

                  b-ifn v. disc    -1.3969      0.3732
```

Note that the variance associated with mothers has been reduced from .9488 to .7107, a reduction of 25%

$$1-\frac{\hat{\tau}_{00C}}{\hat{\tau}_{00U}}=1-\frac{.71}{.95}=.25$$

The omnibus test of the group effect is significant, indicating that the average birth weights of the three groups differ in the population. More detail on this effect can be gleaned from the coefficient estimates in the “Solution for Fixed Effects” table. These intercept estimate indicates that the average birth weight for an infant born to a healthy mother is 8.3001 lbs. In comparison, the average birth weight for an infant of a mother with MS who takes beta-interferon is l.2688 lbs lower. The average birth weight for an infant of a mother with MS who stopped taking beta-interferon prior to conception is .7570 lbs lower than the weight of infants of healthy mothers, a marginal difference.

The way the group variable was coded did not provide a comparison of the b-ifn and disc groups, hence we used the ESTIMATE statement to compute this contrast. The results indicate that the mean for the b-ifn group is .5118 units lower than the mean for the disc group, but that this difference is not statistically significant.

We will now evaluate whether these effects are maintained after including maternal weight gain and gestational age as covariates.

Adding Lower-Level Predictors to the Model

The covariates will be centered at the median values observed for healthy controls and entered at Level 1 as follows:

Level 1:

$$CWeight\_lb_{ij}=\beta_{0j}+\beta_{1j}GestAge_{ij}+\beta_{2j}MGain\_lb+r_{ij} \qquad r_{ij}\sim N\left(0,\sigma^2\right)$$

Level 2:

$$\beta_{0j}=\gamma_{00}+\gamma_{01}B\text{-}Ifn+\gamma_{02}Disc+u_{0j} \qquad u_{0j}\sim N\left(0,\tau_{00}\right)$$

$$\beta_{1j}=\gamma_{10}$$

$$\beta_{2j}=\gamma_{20}$$

Reduced-Form:

$$CWeight\_lb_{ij} = \gamma_{00} + \gamma_{01}B\text{-}Ifn + \gamma_{02}Disc + \gamma_{10}GestAge_{ij} + \gamma_{20}MGain\_lb + u_{0j} + r_{ij}$$

To fit the model we must first perform the centering. The following SAS code computes the medians for healthy controls and then centers the covariates at those values (centering was performed with respect to the median given a couple of unusually high levels of maternal weight gain among healthy controls). By centering, the intercepts can be interpreted as the expected birth weight for an infant born at a typical gestational age to a mother who experienced the typical amount of weight gain for a healthy pregnancy.

```
proc means data=demo.beta N mean median min max; where group="HCntrl";
 var GestAge MGain_lb;
run;
data beta2; set demo.beta;
  GestAge = GestAge - 38.5;
  MGain_lb = MGain_lb - 37.5;
run;
```

```
                             The MEANS Procedure

 Variable     N           Mean          Median         Minimum         Maximum
 ──────────────────────────────────────────────────────────────────────────────
 GestAge     18     38.2222222      38.5000000      35.0000000      39.0000000
 MGain_lb    18     44.9444444      37.5000000       9.0000000     118.0000000
 ──────────────────────────────────────────────────────────────────────────────
```

We now fit the model as follows:

```
proc mixed data=beta2 method=reml noclprint;
  class MID Group;
  model CWeight_lb = GestAge MGain_lb Group / solution alpha=.05
                     ddfm=kr;
  random intercept / subject=MID v vcorr;
  estimate "b-ifn v. disc" group 1 -1 0 / e cl;
run;
```

Abridged output is shown here:

```
                           Number of Observations

                Number of Observations Read             46
                Number of Observations Used             44
                Number of Observations Not Used          2
```

Note the loss of two observations due to missing data on the maternal weight gain covariate.

```
                      Covariance Parameter Estimates

                     Cov Parm      Subject    Estimate

                     Intercept     MID          0.3291
                     Residual                   0.3323
```

Note that these variance estimates can only roughly be compared to the corresponding estimates from previous models given the difference in sample size (loss of observations due to missing data). The between-mother variance is made smaller by including the Level 1 covariates (due to between-mother differences in maternal weight gain and gestational age). The within-mother variance is similar to before (actually appearing to increase a bit, but bear in mind that these estimates are not very stable given the small sample, and that the sample is not quite the same as before).

```
                        Solution for Fixed Effects

                                Standard
 Effect      group   Estimate      Error    DF  t Value  Pr > |t|   Alpha

 Intercept             8.3364     0.1993  31.3    41.82    <.0001    0.05
 GestAge               0.2734    0.06965  30.7     3.93    0.0005    0.05
 MGain_lb            0.008817   0.006631  31.4     1.33    0.1932    0.05
 group       B-Ifn    -0.9902     0.3400  30.6    -2.91    0.0066    0.05
 group       Disc     -0.1695     0.3537  20.4    -0.48    0.6369    0.05
 group       HCntrl         0          .     .        .         .       .

                        Solution for Fixed Effects

                Effect      group       Lower       Upper

                Intercept              7.9299      8.7428
                GestAge                0.1313      0.4155
                MGain_lb             -0.00470     0.02233
                group       B-Ifn     -1.6840     -0.2964
                group       Disc      -0.9064      0.5674
                group       HCntrl          .           .


                      Type 3 Tests of Fixed Effects

                            Num     Den
             Effect         DF      DF    F Value    Pr > F

             GestAge         1    30.7      15.41    0.0005
             MGain_lb        1    31.4       1.77    0.1932
             group           2    23.4       4.57    0.0211
```

The omnibus test of the group effect is still significant. Of interest, the difference between the Disc and HCntrl groups has largely vanished. The difference between the B-Ifn and HCntrl group is smaller but still statistically significant.

```
                              Estimates

                          Standard
 Label           Estimate      Error     DF   t Value   Pr > |t|    Alpha

 b-ifn v. disc    -0.8207     0.3738   20.9     -2.20     0.0396     0.05

                              Estimates

                  Label             Lower       Upper

                  b-ifn v. disc    -1.5983    -0.04309
```

The difference between the B-Ifn and Disc groups is now statistically significant.

Within SAS, all pair-wise comparisons between levels of a CLASS variable can also be obtained another way, as shown in the code below:

```
proc mixed data=beta2 method=reml noclprint;
  class MID Group;
  model CWeight_lb = GestAge MGain_lb Group / solution alpha=.05
                   ddfm=kr;
  random intercept / subject=MID;
  lsmeans Group / at (GestAge MGain_lb)=(0 0) diff adjust=Tukey;
  ods output lsmeans=adjgroupmeans;
run;
```

Syntax Notes:

- The LSMEANS statement: Used to compute "least squares" means generates conditional mean estimates, adjusted for the covariates.
    - The AT option is used to hold the covariates at specific values (the default is to hold them at their means). Here, they are held at zero, the median values for the healthy control group after centering.
    - The DIFF option requests tests of mean differences between pairs of levels of the CLASS variable.
    - The ADJUST option requests adjusted significance testing so that the family-wise α-level will be maintained at .05. This is an important advantage of the LSMEANS statement relative to the ESTIMATE statement, particularly when the classification variable has more than a few levels. Here, the Tukey-Kramer adjustment has been requested (other options include Bonferroni, Scheffe, Dunnett, etc)
- The ODS statement: Calls the Output Delivery System and is used for a variety of purposes.
    - ODS OUTPUT tells SAS to convert a table of output into an active dataset. This statement is followed by a key word for a table of output ("lsmeans") and then the name of the dataset to be created ("adjgroupmeans"). In the present case, the conditional means are being output in order to graph them. Keywords for tables can be found in the user guide documentation, online help, or by using ODS TRACE.

The new output produced by LSMEANS is shown here:

```
                           Least Squares Means

                    Gest                     Standard
Effect   group       Age   MGain_lb   Estimate      Error     DF   t Value

group    B-Ifn      0.00       0.00     7.3462     0.2703   28.9     27.18
group    Disc       0.00       0.00     8.1668     0.2948     16     27.70
group    HCntrl     0.00       0.00     8.3364     0.1993   31.3     41.82

                           Least Squares Means

                        Effect   group    Pr > |t|

                        group    B-Ifn      <.0001
                        group    Disc       <.0001
                        group    HCntrl     <.0001
                    Differences of Least Squares Means

                          Gest                      Standard
Effect  group   _group     Age  MGain_lb  Estimate     Error    DF  t Value

group   B-Ifn   Disc      0.00      0.00   -0.8207    0.3738  20.9    -2.20
group   B-Ifn   HCntrl    0.00      0.00   -0.9902    0.3400  30.6    -2.91
group   Disc    HCntrl    0.00      0.00   -0.1695    0.3537  20.4    -0.48

                    Differences of Least Squares Means

        Effect  group   _group  Pr > |t|    Adjustment       Adj P

        group   B-Ifn   Disc      0.0396    Tukey-Kramer    0.0929
        group   B-Ifn   HCntrl    0.0066    Tukey-Kramer    0.0204
        group   Disc    HCntrl    0.6369    Tukey-Kramer    0.8817
```

The conditional means (adjusted for the covariates) are 7.3462, 8.1668, 8.3364 for the B-Ifn, Disc and HCntrl groups, respectively. Note that after adjustment for multiple comparisons, the B-Ifn versus Disc difference is non-significant. The conditional means are now in the dataset **`adjgroupmeans`**. This data set is shown here:

```
proc print data=adjgroupmeans;
run;
```

```
                      Gest
  Obs Effect group     Age MGain_lb Estimate   StdErr   DF  tValue  Probt

   1  group  B-Ifn    0.00    0.00    7.3462   0.2703 28.9   27.18 <.0001
   2  group  Disc     0.00    0.00    8.1668   0.2948   16   27.70 <.0001
   3  group  HCntrl   0.00    0.00    8.3364   0.1993 31.3   41.82 <.0001
```

We can now plot these means as shown here:

```
proc gchart data=adjgroupmeans;
 vbar group /discrete type=mean sumvar=estimate axis=axis1 descending;
 pattern value=solid color=red;
 axis1 label=("CWeight_lb") minor=none value=("0" "1" "2" "3" "4" "5"
                                            "6" "7" "8" "9");
run; quit;
```

The resulting plot can be compared to the prior plot of unadjusted (raw) group means to see the impact of controlling for the covariates.