

Session 10: Repeated Measures and Longitudinal Analysis II

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CUNY SPH Biostatistics 2

Learning objectives and outline

Learning objectives

- 1 Define mixed effects models and population average models
- 2 Perform model diagnostics for random effects models
- 3 Interpret random intercepts and random slopes
- 4 Define and perform population average models
- 5 Define assumptions on correlation structure in hierarchical models
- 6 Choose between hierarchical modeling strategies

Outline

- 1 Review of fecal fat dataset
 - 2 Summary of non-hierarchical approaches
 - 3 Mixed effects models
 - 4 Longitudinal data and the Georgia Birthweights dataset
 - 5 Population average models and Generalized Estimating Equations (GEE)
- Vittinghoff sections 7.2, 7.3, 7.5

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Learning
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Review

Non-
hierarchical
analysis
strategies

Mixed effects
models

Longitudinal
data

Population
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Models

Review

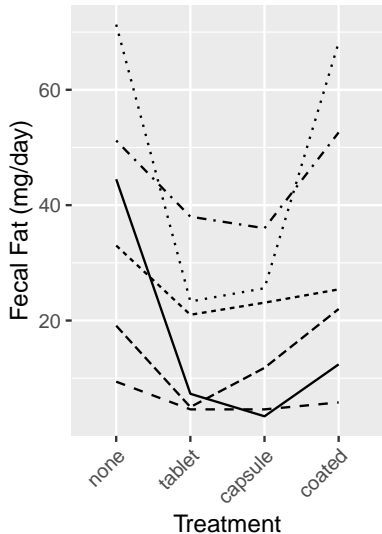
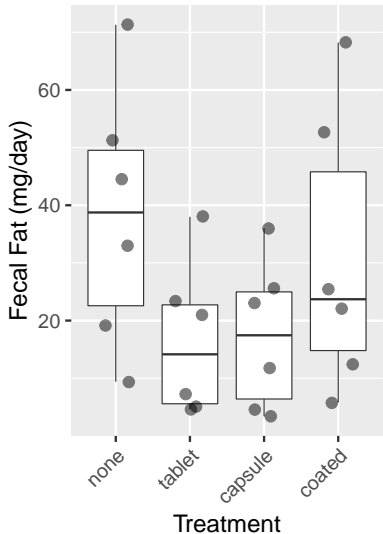
Fecal fat dataset

- Lack of digestive enzymes in the intestine can cause bowel absorption problems.
 - This will be indicated by excess fat in the feces.
 - Pancreatic enzyme supplements can alleviate the problem.
 - fecfat.csv: a study of fecal fat quantity (g/day) for individuals given each of a placebo and 3 types of pills

Table 7.1 Fecal fat (g/day) for six subjects

Subject number	Pill type				Subject Average
	None	Tablet	Capsule	Coated	
1	44.5	7.3	3.4	12.4	16.9
2	33.0	21.0	23.1	25.4	25.6
3	19.1	5.0	11.8	22.0	14.5
4	9.4	4.6	4.6	5.8	6.1
5	71.3	23.3	25.6	68.2	47.1
6	51.2	38.0	36.0	52.6	44.5
Pill type average	38.1	16.5	17.4	31.1	25.8

Fecal fat dataset



Analysis strategies for hierarchical data

- Fixed effects and other non-hierarchical strategies
- Random / mixed effects models
 - model certain regression coefficients (intercept, slopes) as random variables
- Population average models
 - using Generalized Estimating Equations (GEE)

Non-hierarchical analysis strategies

Non-hierarchical analysis strategies for hierarchical data

- Analyses for each subgroup
 - e.g., look at each patient independently
 - doesn't work at all in this example, and in general is not an integrated analysis of the whole data
 - could sort of work for an example with many patients per doctor, a few doctors
- Analysis at the highest level in the hierarchy
 - first summarize data to highest level
 - doesn't work at all in this example
 - could sort of work for an example with few patients per doctor, many doctors
- Analysis on "Derived Variables"
 - consider each treatment type separately, take differences in fat levels between treatment/control for each patient and use paired t-tests
 - can work, but not for unbalanced groups
- Fixed-effects models

When is hierarchical analysis definitely needed?

- 1 the correlation structure is of interest, e.g. familial aggregation of disease, or consistency of treatment within centers
- 2 we wish to “borrow strength” across the levels of a hierarchy in order to improve estimates
- 3 dealing with unbalanced data
- 4 we want to benefit from software designed for hierarchical data

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Mixed effects models

Mixed effects models

- Model looks like two-way ANOVA:

$$FECFAT_{ij} = \beta_0 + \beta_{subjecti} SUBJECT_i + \beta_{pilltypej} PILLTYPE_j + \epsilon_{ij}$$

- Assumption: $\epsilon_i \stackrel{iid}{\sim} N(0, \sigma_\epsilon^2)$
- But instead of fitting a β to each individual, we assume that the subject effects are selected from a distribution of possible subject effects:

$$FECFAT_{ij} = \beta_0 + SUBJECT_i + \beta_{pilltypej} PILLTYPE_j + \epsilon_{ij}$$

Where we assume: $SUBJECT_i \stackrel{iid}{\sim} N(0, \tau_{00}^2)$

- This is a *mixed effects* model because:
 - the “true” intercept varies randomly from patient to patient
 - the “true” (population) coefficient of treatment is fixed (the same for everyone)

Fit this mixed-effects model

```
library(nlme)
fitmix <- nlme::lme(fecfat ~ pilltype,
                    data = dat,
                    random = ~ 1 | subject)
```

Note: the lme4 package is another popular alternative

Mixed effects model coefficients, variances, ICC

```
## Linear mixed-effects model fit by REML
## Data: dat
## Log-restricted-likelihood: -84.55594
## Fixed: fecfat ~ pilltype
## (Intercept) pilltypetablet pilltypecapsule pilltypecoated
## 38.083334 -21.550001 -20.666667 -7.016668
##
## Random effects:
## Formula: ~1 | subject
## (Intercept) Residual
## StdDev: 15.89557 10.34403
##
## Number of Observations: 24
## Number of Groups: 6
```

$$ICC = 15.9^2 / (15.9^2 + 10.34^2) = 0.7 = 0.7.$$

- Recall ICC is a measure of how large the subject effect is, in relation to the error term
- Variances were estimated directly by the model!

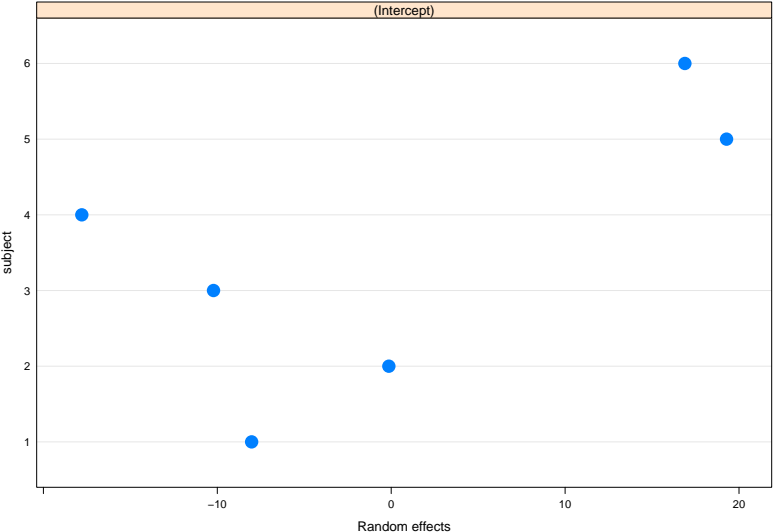
Assumptions of the mixed model

$$FECFAT_{ij} = \beta_0 + SUBJECT_i + \beta_{pilltypej}PILLTYPE_j + \epsilon_{ij}$$

- Normally distributed residuals as in fixed effects model:
 - $\epsilon_i \stackrel{iid}{\sim} N(0, \sigma_\epsilon^2)$
- Normally distributed **latent variable**:
 - $SUBJECT_i \stackrel{iid}{\sim} N(0, \tau_{00}^2)$

Mixed effects model results

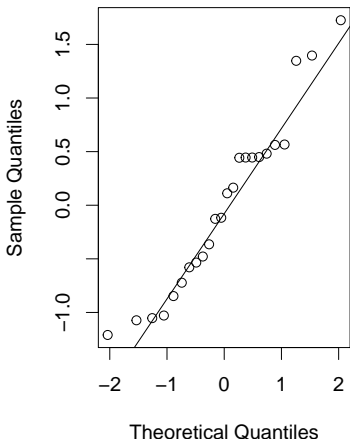
A plot of the random intercept:



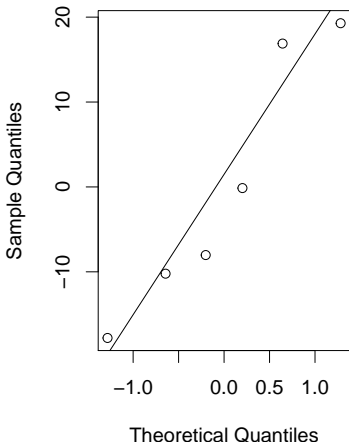
Mixed effects model diagnostics

“

QQ plot residuals



QQ plot random intercepts



Mixed effects model results

```
## Linear mixed-effects model fit by REML
## Data: dat
##      AIC      BIC    logLik
##  181.1119 187.0863 -84.55594
##
## Random effects:
## Formula: ~1 | subject
##      (Intercept) Residual
## StdDev:      15.89557 10.34403
##
## Fixed effects: fecfat ~ pilltype
##              Value Std.Error DF   t-value p-value
## (Intercept)   38.08333   7.742396 15   4.918805  0.0002
## pilltypetablet -21.55000   5.972127 15  -3.608430  0.0026
## pilltypecapsule -20.66667   5.972127 15  -3.460521  0.0035
## pilltypecoated  -7.01667   5.972127 15  -1.174903  0.2583
## Correlation:
##      (Intr) plltypt plltycp
## pilltypetablet -0.386
## pilltypecapsule -0.386  0.500
## pilltypecoated -0.386  0.500  0.500
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.210052934 -0.615068039 -0.002727166  0.457105344  1.725618643
##
## Number of Observations: 24
## Number of Groups: 6
```

- Note: correlation of the estimator of the fixed effects
 - high correlations may (but not necessarily) be due to collinearity

Mixed effects model results

Inference for variance terms (and fixed effects):

```
## Approximate 95% confidence intervals
##
## Fixed effects:
##           lower      est.      upper
## (Intercept)  21.58081  38.08334  54.585860
## pilltypetablet -34.27929 -21.550001 -8.820714
## pilltypecapsule -33.39595 -20.666667 -7.937381
## pilltypecoated -19.74595  -7.016668  5.712618
## attr("label")
## [1] "Fixed effects:"
##
## Random Effects:
## Level: subject
##           lower      est.      upper
## sd((Intercept)) 8.00117 15.89557 31.57904
##
## Within-group standard error:
##           lower      est.      upper
## 7.23240 10.34403 14.79438
```

- Would conclude that variation of the intercept between subjects is non-zero
 - not attributable to within-subject variation

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Longitudinal data

Longitudinal data

- Interested in the change in the value of a variable within a “subject”
- Collect data repeatedly through time.
- For hierarchical longitudinal analysis to be effective, before/after measurements need to be positively correlated

Longitudinal data

- Interested in the change in the value of a variable within a “subject”
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- For hierarchical longitudinal analysis to be effective, before/after measurements need to be positively correlated

Longitudinal data examples

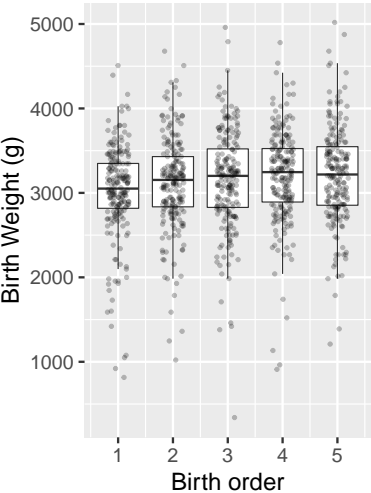
- Example 1: a measure of sleepiness before and after administration of treatment or placebo
- Example 2: Study of Osteoporotic Fractures (SOF dataset)
 - 9,704 women tracked with clinical visits every two years
 - Bone Mineral Density (BMD), Body Mass Index (BMI), many other variables
- Questions for Example 2:
 - 1 Is change in BMD related to age at menopause? This is a time-invariant predictor, age at menopause, with time-dependent changes in the outcome, BMD.
 - 2 Is change in BMD related to change in BMI? This is an analysis relating a time-varying predictor, BMI, with changes in the outcome, BMD. BMI varies quite a lot between women, but also varies within a woman over time.

Longitudinal data examples

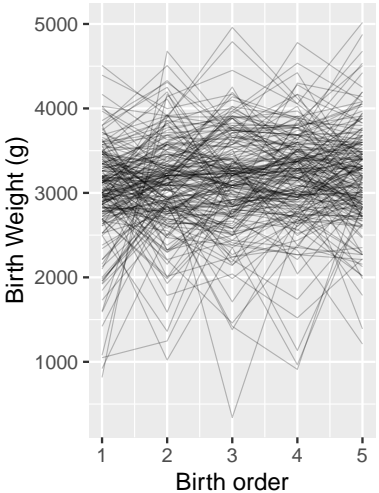
- birthweight and birth order
- provides birthweights and order of infants from mothers who had 5 children in Georgia
 - interested in whether birthweight of babies changes with order
 - whether this difference depends on the *mother's age at first childbirth* or on the *weight of initial baby*.

Georgia Birthweights dataset

Boxplot and “Spaghetti” plot:
Georgia birthweight dataset



Georgia birthweight dataset



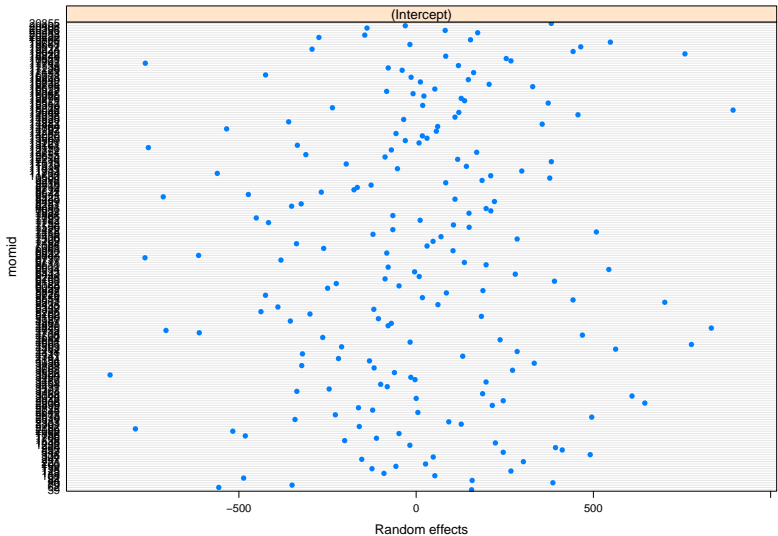
Georgia Birthweights dataset

- Does baseline birth weight vary by mother?
 - random intercept

```
library(nlme)
gafit1 <- lme(bweight ~ birthord, data=ga,
              random=~1|momid)
```

Note: there are not enough degrees of freedom to also fit a random coefficient for birth order

Georgia Birthweights dataset



Georgia Birthweights dataset

```
summary(gafit1)
```

```
## Linear mixed-effects model fit by REML
## Data: ga
##      AIC      BIC    logLik
## 15321.65 15341.28 -7656.826
##
## Random effects:
## Formula: ~1 | momid
##      (Intercept) Residual
## StdDev:      367.2676 445.0228
##
## Fixed effects: bweight ~ birthord
##              Value Std.Error DF t-value p-value
## (Intercept) 2995.640  41.99615 799 71.33130      0
## birthord    46.608   9.95101 799  4.68374      0
## Correlation:
##      (Intr)
## birthord -0.711
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -5.26801358 -0.43683345  0.05028638  0.52703429  3.30770805
##
## Number of Observations: 1000
## Number of Groups: 200
```

Georgia Birthweights dataset

```
intervals(gafit1, which = "all")
```

```
## Approximate 95% confidence intervals
##
## Fixed effects:
##           lower      est.      upper
## (Intercept) 2913.20418 2995.640 3078.07582
## birthord    27.07478   46.608   66.14122
## attr("label")
## [1] "Fixed effects:"
##
## Random Effects:
##   Level: momid
##           lower      est.      upper
## sd((Intercept)) 323.1724 367.2676 417.3794
##
## Within-group standard error:
##           lower      est.      upper
## 423.7298 445.0228 467.3859
```

- Does baseline birth weight vary by mother?
 - yes: the subject variance is significantly greater than zero
 - The variance between mothers is too much to be explained by within-mother variation in birth weights

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Population Average Models

Population Average Models

- An alternative to random / mixed-effects models that is more robust to assumptions of:
 - distribution of random effects
 - correlation structure
- Estimates correlation structure from the data rather than assuming normality
 - Requires more clusters than observations per cluster
- Estimates regression coefficients and robust standard errors
 - commonly by Generalized Estimating Equations (GEE)

Population Average Models

- Compare mixed model multiple linear regression:

$$E[Y_{ij}|X_{ij}] = \beta_0 + \alpha_{0j} + \beta_1 X_{ij}, \alpha_{0j} \sim N(0, \sigma)$$

for subject i in group j .

- to a population average model:

$$E[Y_{ij}|X_{ij}] = \beta_0^* + \beta_1^* X_{ij}$$

- Interpretations of β^* and β are equivalent
- Numerically equivalent for linear and log-linear models (if specification of mixed model is correct), but not for logistic link.

Fit a population average model

```
gafit.gee <- gee::gee(bweight ~ birthord,  
                      corstr = "exchangeable",  
                      id = momid,  
                      data = ga)
```

```
summary(gafit.gee)
```

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```
##
## GEE:  GENERALIZED LINEAR MODELS FOR DEPENDENT DATA
## gee S-function, version 4.13 modified 98/01/27 (1998)
##
## Model:
## Link:                               Identity
## Variance to Mean Relation: Gaussian
## Correlation Structure:      Exchangeable
##
## Call:
## gee::gee(formula = bweight ~ birthord, id = momid, data = ga,
##   corstr = "exchangeable")
##
## Summary of Residuals:
##      Min       1Q   Median       3Q      Max
## -2795.464 -299.126   48.840   341.144  1824.536
##
## Coefficients:
##              Estimate Naive S.E.   Naive z Robust S.E.  Robust z
## (Intercept) 2995.640  41.973695 71.369462   38.808066 77.191170
## birthord     46.608   9.958128  4.680398    9.996256  4.662546
##
## Estimated Scale Parameter:  332525.3
## Number of Iterations:  1
##
## Working Correlation
##           [,1]      [,2]      [,3]      [,4]      [,5]
## [1,] 1.0000000 0.4035684 0.4035684 0.4035684 0.4035684
## [2,] 0.4035684 1.0000000 0.4035684 0.4035684 0.4035684
## [3,] 0.4035684 0.4035684 1.0000000 0.4035684 0.4035684
## [4,] 0.4035684 0.4035684 0.4035684 1.0000000 0.4035684
## [5,] 0.4035684 0.4035684 0.4035684 0.4035684 1.0000000
```

Correlation assumptions for GEE

Must make some assumption about the form of correlation among grouped observations. Some options are:

- Independence:
 - no correlation between measurements within group
- Exchangeable:
 - all pairwise correlations are the same (in large-N limit)
 - nothing distinguishes one member of a cluster from another
 - appropriate in the absence of other data structures such as measurements taken through time or space
- Auto-regressive ($AR-M$):
 - observations taken more closely in time are more highly correlated

Correlation assumptions for GEE (cont'd)

- Unstructured:
 - estimates a separate correlation between observations taken on each pair of “times”
- Non-stationary (“non_stat_M_dep”):
 - similar to unstructured, but assumes all correlations for pairs separated far enough in time are zero
- Stationary (“stat_M_dep”):
 - e.g. stationary of order 2: observations taken at time points 1 and 3 have the same correlation as time points 2 and 4
 - but this might be different from the correlation between observations taken at times 2 and 3
 - correlations for observations 3 or more time periods apart assumed to be zero

Fewer assumptions requires more data, and good assumptions improve results

Help in choosing a method

Characteristic	Marginal	Fixed-effect	Mixed-effect
Distinguishes observations belonging to the same or different subjects	Yes ^a	Yes	Yes
Reliant on distribution of subject-specific effects	No	No	Yes
Subjects considered a sample from a population larger than the sample itself	Yes ^a	No	Yes
Computation handles few subjects well	No	Yes	No
Computation handles a very large number of subjects well	Yes	No	Yes
Noisy for few observations per subject	No	Yes	No
Computation handles a large number of observations per subject	Depends ^b	Yes	Yes
Accommodates variable observations per subject	Yes	Yes	Yes

Note: ^aOnly for calculation of standard errors.

^bProblems can arise under some specifications of the working covariance structure and depending on the estimation method used.

doi:10.1371/journal.pone.0146721.t002

Figure 2: Hierarchical modeling decision table from Moen *et al.*

Conclusions

- Ignoring within-subject correlations can produce very wrong results, and is not always “conservative”
- Hierarchical analysis strategies are needed for any of:
 - ① When the correlation structure is of primary interest, e.g. familial aggregation of disease, or consistency of treatment within centers,
 - ② When we wish to “borrow strength” across the levels of a hierarchy in order to improve estimates, and
 - ③ When dealing with unbalanced correlated data. E.g., no requirement that each Georgia mother have exactly 5 children.
- Population average models provide a robust alternative to mixed models
 - for one level of hierarchy

A final note on reporting results of hypothesis tests

- Include test statistic, a measure of “effect size”, and test name if unclear from test statistic
- Write in plain language and let the statistics support, not lead. E.g.:
 - *do*: The 36 study participants had a mean age of 27.4 (SD = 12.6), significantly older than the university mean of 21.2 years ($t(35) = 2.95, p = 0.01$).
 - *don't*: A p-value of 0.01 indicated significant difference in age of study participants compared to all university students.
 - *do*: report confidence intervals where possible
- UW “Reporting Results of Common Statistical Tests in APA Format”: specific examples of reporting a hypothesis test result
- STROBE guidelines for reporting observational studies: <https://www.strobe-statement.org/>
- A Guideline for Reporting Results of Statistical Analysis in Japanese Journal of Clinical Oncology: helpful guidelines for all parts of a manuscript

CONGRATULATIONS!!!

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