Repeated Measures ANOVA

Repeated measures by profile analysis

- More than one response measurement for each subject. Might be
 - measurements of the same thing at different times
 - measurements of different but related things
- Generalization of matched pairs ("matched triples", etc.).
- Variation: each subject does several different treatments at different times (called *crossover design*).
- Expect measurements on same subject to be correlated, so assumptions of independence will fail.
- Called *repeated measures*. Different approaches, but *profile analysis* uses Manova (set up right way).
- Another approach uses mixed models (random effects).

Packages

```
library(car)
library(tidyverse)
library(lme4) # for mixed models later
```

Example: histamine in dogs

- 8 dogs take part in experiment.
- Dogs randomized to one of 2 different drugs.
- Response: log of blood concentration of histamine 0, 1, 3 and 5 minutes after taking drug. (Repeated measures.)
- Data in dogs.txt, column-aligned.

Read in data

```
my url <- "http://ritsokiguess.site/datafiles/dogs.txt"
dogs <- read table(my url)</pre>
##
## -- Column specification
## cols(
##
     dog = col_character(),
##
     drug = col_character(),
##
     x = col character(),
##
     lh0 = col double(),
     lh1 = col double(),
##
##
     lh3 = col double(),
     lh5 = col double()
##
## )
```

Setting things up

dogs

dog	drug	X	lh0	lh1	lh3	lh5
A	Morphine	N	-3.22	-1.61	-2.30	-2.53
В	Morphine	Ν	-3.91	-2.81	-3.91	-3.91
C	Morphine	Ν	-2.66	0.34	-0.73	-1.43
D	Morphine	Ν	-1.77	-0.56	-1.05	-1.43
Ε	Trimethaphan	Ν	-3.51	-0.48	-1.17	-1.51
F	Trimethaphan	Ν	-3.51	0.05	-0.31	-0.51
G	Trimethaphan	Ν	-2.66	-0.19	0.07	-0.22
Н	Trimethaphan	Ν	-2.41	1.14	0.72	0.21

```
response <- with(dogs, cbind(lh0, lh1, lh3, lh5))
dogs.1 <- lm(response ~ drug, data = dogs)
response</pre>
```

The repeated measures MANOVA

Get list of response variable names; we call them times. Save in data frame.

```
times <- colnames(response)
times

## [1] "lh0" "lh1" "lh3" "lh5"
times.df <- data.frame(times=factor(times))
times.df</pre>
```

```
\frac{\text{times}}{\text{lh0}} lh1 lh3 lh5
```

```
dogs.2 <- Manova(dogs.1,
idata = times df</pre>
```

The output (some; there is a lot)

summary (dogs.2)

```
##
## Type II Repeated Measures MANOVA Tests:
## Term: (Intercept)
##
   Response transformation matrix:
      (Intercept)
## 1h0
## 1h1
## 1h3
## 1h5
## Sum of squares and products for the hypothesis:
             (Intercept)
                285.366
## (Intercept)
##
## Multivariate Tests: (Intercept)
                 Df test stat approx F num Df den Df Pr(>F)
## Pillai
              1 0.763467 19.36642 1 6 0.0045648 **
            1 0.236533 19.36642 1 6 0.0045648 **
## Wilks
## Hotelling-Lawley 1 3.227738 19.36642 1 6 0.0045648 **
## Rov
        1 3.227738 19.36642 1 6 0.0045648 **
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
```

Term: drug

What there is here

- three sets of tests, for
 - times
 - drug
 - their interaction
- two types of test for each of these:
 - multivariate
 - univariate
- multivariate is the same as MANOVA
- univariate is more powerful if it applies

Sphericity

- The thing that decides whether the univariate tests apply is called "sphericity".
- This holds if the outcomes have equal variance (to each other) and have the same (positive) correlation across subjects.
- Tested using Mauchly's test (part of output)
- If sphericity rejected, there are adjustments to the univariate P-values due to Huynh-Feldt and Greenhouse-Geisser. Huynh-Feldt better if responses not actually normal (safer). Or use multivariate test.

Univariate tests

```
summary(dogs.2)$sphericity.tests
           Test statistic p-value
##
## times
                 0.12334 0.084567
## drug:times
                0.12334 0.084567
summary(dogs.2)$pval.adjustments
##
              GG eps Pr(>F[GG]) HF eps Pr(>F[HF])
## times
           0.5261798 3.744618e-06 0.6822614 1.843418e-07
## drug:times 0.5261798 2.348896e-03 0.6822614 7.307096e-04
## attr(."na.action")
## (Intercept)
                  drug
##
## attr(,"class")
## [1] "omit"
summary(dogs.2)$univariate.tests
            Sum Sq num Df Error SS den Df F value Pr(>F)
##
```

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times 26.160 3 2.2534 18 69.6546 4.215e-10 ***
drug:times 5.111 3 2.2534 18 13.6095 7.050e-05 ***

drug 11.520 1 22.1026 6 3.1272 0.127406

Comments

- The sphericity test for the interaction is almost significant
- The H-F adjusted P-value for the interaction is a bit bigger than the univariate one, but still strongly significant.
- Therefore any lack of sphericity does not affect our conclusion: there is an interaction between drug and time
- ie that the effect of time on log-histamine is different for the two drugs.

The multivariate test for interaction

summary(dogs.2)\$multivariate.tests\$`drug:times`

```
##
## Response transformation matrix:
      times1 times2 times3
## 1h0
## lh1
## 1h1 0 1 0
## 1h3 0 0 1
## lh5 -1 -1
##
## Sum of squares and products for the hypothesis:
          times1 times2
                              times3
## times1 7.60500 2.0572500 -0.0292500
## times2 2.05725 0.5565125 -0.0079125
## times3 -0.02925 -0.0079125 0.0001125
##
## Sum of squares and products for error:
                   times2
##
          times1
                            times3
## times1 1.34480 -0.311350 -0.362450
## times2 -0.31135 1.131975 0.346000
## times3 -0.36245 0.346000 0.309175
##
## Multivariate Tests: drug:times
                  Df test stat approx F num Df den Df Pr(>F)
## Pillai
                1 0.894761 11.33619 3 4 0.020023 *
## Wilks
                   1 0.105239 11.33619 3 4 0.020023 *
## Hotelling-Lawley 1 8.502141 11.33619 3 4 0.020023 *
               1 8.502141 11.33619 3 4 0.020023 *
## Roy
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

Comments

- The (appropriately adjusted) univariate test and the multivariate test should give similar results.
- Here, univariate with Huynh-Feldt P-value was 0.00073, multivariate was 0.0200, rather different but both significant.
- Significant interaction is the conclusion here.
- If the interaction had not been significant:
 - cannot remove interaction with time
 - so look at univariate (better, especially if adjusted for sphericity) or multivariate tests of main effects in this model

Next

- Interaction significant. Pattern of response over time different for the two drugs.
- Want to investigate interaction.

The wrong shape

But data frame has several observations per line ("wide format"):
 dogs %>% slice(1:6)

dog	drug	X	lh0	lh1	lh3	lh5
A	Morphine	N	-3.22	-1.61	-2.30	-2.53
В	Morphine	Ν	-3.91	-2.81	-3.91	-3.91
C	Morphine	Ν	-2.66	0.34	-0.73	-1.43
D	Morphine	Ν	-1.77	-0.56	-1.05	-1.43
E	Trimethaphan	Ν	-3.51	-0.48	-1.17	-1.51
F	Trimethaphan	Ν	-3.51	0.05	-0.31	-0.51

- Plotting works with data in "long format": one response per line.
- The responses are log-histamine at different times, labelled lh-something. Call them all lh and put them in one column, with the time they belong to labelled.

Running pivot_longer, try 1

dog	drug	×	time	lh
A	Morphine	N	lh0	-3.22
Α	Morphine	Ν	lh1	-1.61
Α	Morphine	Ν	lh3	-2.30
Α	Morphine	Ν	lh5	-2.53
В	Morphine	Ν	lh0	-3.91
В	Morphine	Ν	lh1	-2.81
В	Morphine	Ν	lh3	-3.91
В	Morphine	Ν	lh5	-3.91
C	Morphine	Ν	lh0	-2.66
C	Morphine	Ν	lh1	0.34
C	Morphine	Ν	lh3	-0.73
C	Morphine	Ν	lh5	-1.43
D	Morphine	Ν	lh0	-1.77
D	Morphine	Ν	lh1	-0.56
D	Morphine	Ν	lh3	-1.05
D	Mornhine Repeated Me	N asures AN	lh5 OVA	_1 43

Getting the times

Not quite right: for the times, we want just the numbers, not the letters 1h every time. Want new variable containing just number in time: parse_number.

dog	drug	Х	timex	lh	time
A	Morphine	N	lh0	-3.22	0
Α	Morphine	Ν	lh1	-1.61	1
Α	Morphine	Ν	lh3	-2.30	3
Α	Morphine	Ν	lh5	-2.53	5
В	Morphine	Ν	lh0	-3.91	0
В	Morphine	Ν	lh1	-2.81	1
В	Morphine	N	lh3	-3.91	3
В	Morphine	N	lh5	-3.91	5
C	Morphine	Ν	lh0	-2.66	0
_					

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What I did differently

- I realized that pivot_longer was going to produce something like lh1, which I needed to do something further with, so this time I gave it a temporary name timex.
- This enabled me to use the name time for the actual numeric time.
- This works now, so next save into a new data frame dogs.long.

Saving the pipelined results

dog	drug	X	timex	lh	time		
A	Morphine	Ν	lh0	-3.22	0		
Α	Morphine	Ν	lh1	-1.61	1		
Α	Morphine	Ν	lh3	-2.30	3		
Α	Morphine	Ν	lh5	-2.53	5		
В	Morphine	Ν	lh0	-3.91	0		
В	Morphine	Ν	lh1	-2.81	1		
В	Morphine	Ν	lh3	-3.91	3		
В	Morphine	Ν	lh5	-3.91	5		
C	Morphine	Ν	lh0	-2.66	0		
	Repeated Measures ANOVA						

Interaction plot

```
ggplot(dogs.long, aes(x = time, y = lh,
                          colour = drug, group = drug)) +
  stat_summary(fun = mean, geom = "point") +
  stat summary(fun = mean, geom = "line")
  0 -
                                                                     drug
 -1-
                                                                     Morphine

    Trimethaphan

 -2-
                                        3
                                  time
```

Comments

- Plot mean 1h value at each time, joining points on same drug by lines.
- drugs same at time 0
- after that, Trimethaphan higher than Morphine.
- Effect of drug not consistent over time: significant interaction.

Take out time zero

- Lines on interaction plot would then be parallel, and so interaction should no longer be significant.
- Go back to original "wide" dogs data frame.

```
response <- with(dogs, cbind(lh1, lh3, lh5)) # excl time 0
dogs.1 <- lm(response ~ drug, data = dogs)
times <- colnames(response)
times.df <- data.frame(times=factor(times))
dogs.2 <- Manova(dogs.1,
   idata = times.df,
   idesign = ~times
)</pre>
```

Results (univariate)

```
summary(dogs.2)$sphericity.tests
             Test statistic p-value
##
                    0.57597 0.25176
## times
## drug:times
                    0.57597 0.25176
summary(dogs.2)$pval.adjustments
##
                GG eps Pr(>F[GG]) HF eps Pr(>F[HF])
             0.7022305 0.0003752847 0.8520467 0.0001117394
## times
## drug:times 0.7022305 0.1078608639 0.8520467 0.0942573437
## attr(."na.action")
## (Intercept)
                     drug
##
## attr(,"class")
## [1] "omit"
summary(dogs.2)$univariate.tests
               Sum Sq num Df Error SS den Df F value Pr(>F)
##
```

```
## drug 16.2197 1 20.1874 6 4.8207 0.07053 .
## times 3.3250 2 0.7301 12 27.3251 3.406e-05 ***
## drug:times 0.3764 2 0.7301 12 3.0929 0.08254 .
```

(Intercept) 24.2607 1 20.1874

6 7.2106 0.03628 *

Comments

- sphericity: no problem (P-value 0.25)
- univariate test for interaction no longer significant (P-value 0.082)
- Huynh-Feldt adjusted P-value for interaction very similar, 0.094 (adjustment not really worth doing)
- look at main effects:
 - strong significance of time, even after taking out time 0
 - actually not significant drug effect, despite interaction plot

Multivariate tests, all at once

```
dogs.2
```

```
##
## Type II Repeated Measures MANOVA Tests: Pillai test statistic
## Df test stat approx F num Df den Df Pr(>F)
## (Intercept) 1 0.54582 7.2106 1 6 0.036281 *
## drug 1 0.44551 4.8207 1 6 0.070527 .
## times 1 0.85429 14.6569 2 5 0.008105 **
## drug:times 1 0.43553 1.9289 2 5 0.239390
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

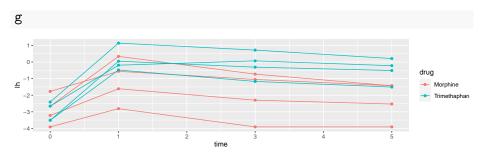
Same conclusions as for univariate tests, though with bigger P-values.

Is the non-significant drug effect reasonable?

- Plot actual data: 1h against days, labelling observations by drug: "spaghetti plot".
- Uses long data frame (confusing, yes I know):
- Plot (time,lh) points coloured by drug
- and connecting measurements for each dog by lines.
- This time, we want group=dog (want the measurements for each dog joined by lines), but colour=drug:

```
g <- ggplot(dogs.long, aes(
    x = time, y = lh,
    colour = drug, group = dog
)) +
    geom_point() + geom_line()</pre>
```

The spaghetti plot



Comments

- For each dog over time, there is a strong increase and gradual decrease in log-histamine. This explains the significant time effect.
- The pattern is more or less the same for each dog, regardless of drug. This explains the non-significant interaction.
- Most of the trimethaphan dogs (blue) have higher log-histamine throughout (time 1 and after), and some of the morphine dogs have lower.
- But two of the morphine dogs have log-histamine profiles like the trimethaphan dogs. This ambiguity is probably why the drug effect is not quite significant.

Mixed models

- Another way to fit repeated measures
- Subjects (on whom repeated measures taken) are random sample of all possible subjects (random effects)
- Times and treatments are the only ones we care about (fixed effects)
- Use package lme4 function lmer (like lm in some ways)
- Uses long-format "tidy" data

Fitting the model (uses 1me4)

```
# dogs.long including time zero
dogs.3 <- lmer(lh~drug*time+(1|dog), data=dogs.long)</pre>
```

• note specification of random effect: each dog has "random intercept" that moves log-histamine up or down for that dog over all times

What can we drop?

• using drop1:

drop1(dogs.3,test="Chisq")

	npar	AIC	LRT	Pr(Chi)
	NA	113.2597	NA	NA
drug:time	1	114.2130	2.953368	0.0856988

• Interaction again not significant, but P-value smaller than before

Re-fit without interaction

```
dogs.4 <- update(dogs.3,.~.-drug:time)
drop1(dogs.4,test="Chisq")</pre>
```

	npar	AIC	LRT	Pr(Chi)
	NA	114.2130	NA	NA
drug	1	115.5691	3.356021	0.0669597
time	1	114.9631	2.750099	0.0972484

- This time neither drug nor (surprisingly) time is significant.
- MANOVA and 1mer methods won't agree, but both valid ways to approach problem.

The exercise data

- 30 people took part in an exercise study.
- Each subject was randomly assigned to one of two diets ("low fat" or "non-low fat") and to one of three exercise programs ("at rest", "walking", "running").
- There are $2 \times 3 = 6$ experimental treatments, and thus each one is replicated 30/6 = 5 times.
- Nothing unusual so far.
- However, each subject had their pulse rate measured at three different times (1, 15 and 30 minutes after starting their exercise), so have repeated measures.

Reading the data

Separated by tabs:

Rows: 90 Columns: 5

```
url <- "http://ritsokiguess.site/datafiles/exercise2.txt"
exercise.long <- read_tsv(url)</pre>
```

```
## -- Column specification -----
## Delimiter: "\t"
## chr (3): diet, exertype, time
## dbl (2): id, pulse
##
## i Use `spec()` to retrieve the full column specification for
```

i Specify the column types or set `show col types = FALSE`

The data (some)

exercise.long %>% slice(1:8)

id	diet	exertype	pulse	time
1	nonlowfat	atrest	85	min01
1	nonlowfat	atrest	85	min15
1	nonlowfat	atrest	88	min30
2	nonlowfat	atrest	90	min01
2	nonlowfat	atrest	92	min15
2	nonlowfat	atrest	93	min30
3	nonlowfat	atrest	97	min01
3	nonlowfat	atrest	97	min15

- This is "long format", which is usually what we want.
- But for repeated measures analysis, we want wide format!
- pivot_wider.

Making wide format

 pivot_wider needs: a column that is going to be split, and the column to make the values out of:

id	diet	exertype	min01	min15	min30
2	nonlowfat	atrest	90	92	93
24	nonlowfat	running	87	132	120
5	nonlowfat	atrest	91	92	91
16	lowfat	walking	84	86	89
27	lowfat	running	100	126	140

 Normally pivot_longer min01, min15, min30 into one column called pulse labelled by the number of minutes. But Manova needs it the other way.

Setting up the repeated-measures analysis

Make a response variable consisting of min01, min15, min30:
 response <- with(exercise.wide, cbind(min01, min15, min30))

• Predict that from diet and exertype and interaction using lm:

```
exercise.1 <- lm(response ~ diet * exertype,
   data = exercise.wide
)</pre>
```

Run this through Manova:

Sphericity tests

```
summary(exercise.2)$sphericity.tests
```

exertype:times 0.92416 0.40372

diet:exertype:times 0.92416 0.40372

No problem with sphericity; go to univariate tests.

Univariate tests

summary(exercise.2)\$univariate.tests

```
## Warning in summary. Anova.mlm(exercise.2): HF eps > 1 treated as 1
##
                     Sum Sq num Df Error SS den Df
                                                   F value
                                                            Pr(>F)
                                   2085.2
                                             24 10296.6595 < 2.2e-16 ***
## (Intercept)
                    894608
## diet
                      1262
                                   2085.2
                                             24
                                                   14.5238 0.0008483 ***
                      8326
                                   2085.2
                                             24 47.9152 4.166e-09 ***
## exertype
## diet:exertype
                       816
                                   2085.2
                                             24 4.6945 0.0190230 *
## times
                      2067
                                   1563.6
                                             48
                                                   31.7206 1.662e-09 ***
## diet:times
                      193
                                   1563.6
                                             48
                                                    2.9597 0.0613651 .
                                   1563.6
                                                   20.9005 4.992e-10 ***
## exertype:times
                      2723
                                             48
## diet:exertype:times
                     614
                                   1563.6
                                             48
                                                    4.7095 0.0027501 **
## ---
## Signif. codes:
                 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

- The three-way interaction is significant
 - the effect of diet on pulse rate over time is different for the different exercise types

Multivariate very similar

```
exercise.2
```

```
##
  Type II Repeated Measures MANOVA Tests: Pillai test statistic
##
                     Df test stat approx F num Df den Df
                         0.99767 10296.7
                                                    24 < 2.2e-16 ***
  (Intercept)
## diet
                         0.37701
                                     14.5
                                                   24 0.0008483 ***
                      2 0.79972 47.9
                                              2
                                                    24 4.166e-09 ***
## exertype
                      2 0.28120 4.7
## diet:exertype
                                                    24 0.0190230 *
## times
                        0.78182 41.2
                                                    23 2.491e-08 ***
                      1 0.25153 3.9
                                                    23 0.0357258 *
## diet:times
                     2 0.83557 8.6
                                              4
                                                    48 2.538e-05 ***
## exertype:times
                      2 0.51750
                                 4.2
                                              4
                                                    48 0.0054586 **
## diet:exertype:times
## ---
## Signif. codes:
                 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

- Three-way interaction significant, so cannot remove anything.
- Pulse rate depends on diet and exercise type *combination*, and *that* is different for each time.

Making some graphs

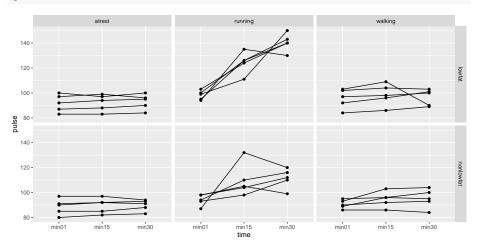
- Three-way interactions are difficult to understand. To make an attempt, look at some graphs.
- Plot time trace of pulse rates for each individual, joined by lines, and make *separate* plots for each diet-exertype combo.
- ggplot again. Using long data frame:

```
g <- ggplot(exercise.long, aes(
  x = time, y = pulse,
  group = id
)) + geom_point() + geom_line() +
  facet grid(diet ~ exertype)
```

• facet_grid(diet~exertype): do a separate plot for each combination of diet and exercise type, with diets going down the page and exercise types going across. (Graphs are usually landscape, so have the factor exertype with more levels going across.) Repeated Measures ANOVA

The graph(s)

g



Comments on graphs

- For subjects who were at rest, no change in pulse rate over time, for both diet groups.
- For walking subjects, not much change in pulse rates over time. Maybe a small increase on average between 1 and 15 minutes.
- For both running groups, an overall increase in pulse rate over time, but the increase is stronger for the lowfat group.
- No consistent effect of diet over all exercise groups.
- No consistent effect of exercise type over both diet groups.
- No consistent effect of time over all diet-exercise type combos.

"Simple effects" of diet for the subjects who ran

- Looks as if there is only any substantial time effect for the runners. For them, does diet have an effect?
- Pull out only the runners from the wide data:

```
exercise.wide %>%
  filter(exertype == "running") -> runners.wide
```

 Create response variable and do MANOVA. Some of this looks like before, but I have different data now:

```
response <- with(runners.wide, cbind(min01, min15, min30))
runners.1 <- lm(response ~ diet, data = runners.wide)
times <- colnames(response)
times.df <- data.frame(times=factor(times))
runners.2 <- Manova(runners.1,
   idata = times.df,
   idesign = ~times
)</pre>
```

Sphericity tests

diet:times

```
summary(runners.2)$sphericity.tests
```

0.81647 0.4918

No problem, look at univariate tests.

Univariate tests

summary(runners.2)\$univariate.tests

```
## Warning in summary.Anova.mlm(runners.2): HF eps > 1 treated as 1

## Sum Sq num Df Error SS den Df F value Pr(>F)

## (Intercept) 383522 1 339.2 8 9045.3333 1.668e-13 ***

## diet 1920 1 339.2 8 45.2830 0.0001482 ***

## times 4714 2 1242.0 16 30.3644 3.575e-06 ***

## diet:times 789 2 1242.0 16 5.0795 0.0195874 *

## ---

## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

- Interaction still significant
 - dependence of pulse rate on time still different for the two diets

Multivariate results (comparison)

```
runners.2
```

```
##

## Type II Repeated Measures MANOVA Tests: Pillai test statistic

## Df test stat approx F num Df den Df Pr(>F)

## (Intercept) 1 0.99912 9045.3 1 8 1.668e-13 ***

## diet 1 0.84986 45.3 1 8 0.0001482 ***

## times 1 0.92493 43.1 2 7 0.0001159 ***

## diet:times 1 0.68950 7.8 2 7 0.0166807 *

## ---

## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

Same.

How is the effect of diet different over time?

• Table of means. Only I need long data for this:

- ## `summarise()` has grouped output by 'time'. You can overric
 ## the `.groups` argument.
 - Result of summarize is data frame, so can save it (and do more with it if needed).

Understanding diet-time interaction

• The summary:

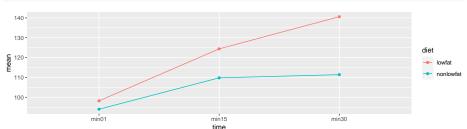
SIIMM

time	diet	mean	sd
min01	lowfat	98.2	3.701351
min01	nonlowfat	94.0	4.527693
min15	lowfat	124.4	8.619745
min15	nonlowfat	109.8	13.122500
min30	lowfat	140.6	7.197222
min30	nonlowfat	111.4	7.924645

- Pulse rates at any given time higher for lowfat (diet effect),
- Pulse rates increase over time of exercise (time effect),
- but the amount by which pulse rate higher for a diet depends on time: diet by time interaction.

Interaction plot

 We went to trouble of finding means by group, so making interaction plot is now mainly easy:



Comment on interaction plot

- The lines are not parallel, so there is interaction between diet and time for the runners.
- The effect of time on pulse rate is different for the two diets, even though all the subjects here were running.