Proceedings
Whole Grains Summit 2012

Crossover Designs in Nutrition: New Methodology Accounting for Individually Varying Responses

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ABSTRACT SUMMARY

Crossover designs are a mainstay in human nutrition research. Traditionally, analyses use ANOVA, with subjects and diets as main effects and the subject–diet interaction serving as the error term (since subjects do not replicate diets). If subjects do not respond to diets in the same way, then the subject–diet interaction term is large, the ANOVA model is misspecified, and the test on the diet main effect becomes too liberal. Nonetheless, in the more than 40,000 nutritional studies using crossover designs done since 2000, none estimated the potentially important subject–diet interaction.

A multiplicative (singular value or principal components) decomposition of the "residual" is proposed, which separates the subject–diet interaction from error. The method is demonstrated using a recent crossover study and then compared with a second study where subjects repeated some diets to allow for an independent estimate of error. In other data sets available to us, over half of the dependent variables had significant subject–diet interactions.

Introduction

While the many peculiarities of crossover designs, e.g., period and carry-over effects, have been researched (8), estimating subject–diet interactions has been neglected. Traditionally these designs are not replicated (subjects do not repeat diets) and analyzed in an ANOVA framework. Without replication, the subject–diet interaction is confounded with the residual term.

A search through the literature (over 40,000 studies from 2000–2009, inclusive) for subject–diet interactions in human nutrition studies yielded no "hits," which is surprising because researchers seem to be well aware of the heterogeneity in responsiveness to dietary interventions (12). The lack of hits is consistent with our subjective opinion that researchers do not recognize that a subject by diet interaction term is missing in their statistical models. In general, misspecifying the model in this way makes the test on diet too liberal, especially in mixed models (where subject, and by extension, the subject–diet interaction, are random effects), discussed in Boykin et al. (2).

Some in the field suspect that the subject–diet interaction can be large and should be accounted for in clinical trials (7). One technique to estimate this term for a single dependent variable used subject clusters (6). However, for multiple dependent variables we found that subject group compositions change for different collections of dependent variables, impacting the estimates of the interaction and residual terms.

A different approach is to use a multiplicative decomposition of a "residual" formed by subtracting estimated main effects from the data to extract the subject–diet interaction component. In this paper, we outline this method for a nonreplicated human nutrition study. We do a similar analysis for a second study where there was some replication, allowing us to directly compare this method with an analysis using traditional ANOVA methods. We then briefly discuss results from the first data set and a third data set, both with many different dependent variables, to estimate how often a subject–diet interaction occurred.

Description and Analysis of Dataset 1

An analysis of these data was published in Chen et al. (3). The objective of the study was to investigate the interaction

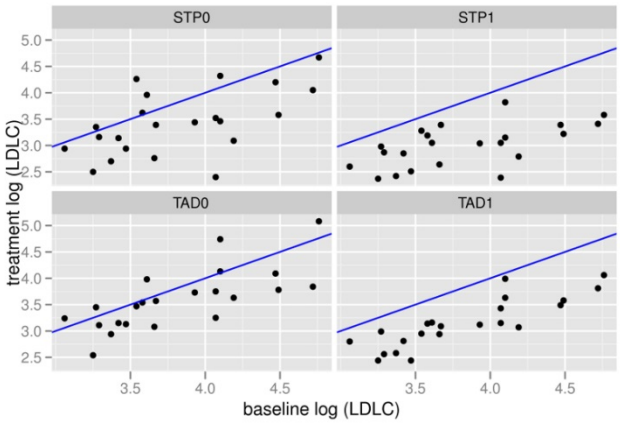

Fig. 1. Scatter plots of pre-experiment baseline log LDL-cholesterol values versus log LDL-cholesterol values on each of the four diets. The blue line represents no change from baseline. STP = Step1, or TAD = typical American diet; 1 = plant sterols added, or 0 = not, to diet.

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http://dx.doi.org/10.1094/CPLEX-2013-1001-03B

between diet (typical American diet versus Step-1 diet) and consuming plant sterols (0 and 3.3 g/day) on cholesterol. While a number of blood compounds were measured on the 22 adults, we only discuss results for LDL-cholesterol. Each subject consumed each of the four diets, though the order differed. Measures are means of two samples, from day 22 and day 24 of each period. Baseline (pre-experiment) measurements were taken during the week prior to the beginning of the experiment. In this analysis we took natural logs of LDL-cholesterol. Figure 1 gives scatter plots representing each subject on each of the four diets; the blue line in each plot represents no change from baseline. What is obvious from this figure is the large effect of adding plant sterols to a diet (second column).

The ANOVA residuals contain the confounded within-subject error and diet–treatment interaction effects. An AMMI (additive main effects, multiplicative interaction) model does a singular value (principal components) decomposition of the residuals, after they have been arrayed into a subject (row) by diet (column) matrix. Typically, the first (or first and second) principal component(s) are used to capture the interaction; the remainder of the variance is attributed to within-subject error. The AMMI model can be written as follows:

$$y_{ijk} - \left(\hat{\mu} + \hat{\boldsymbol{\beta}}\boldsymbol{x}_j + \hat{\tau}_i + \hat{\gamma}_j\right) = \sum_r \lambda_r v_{ir}\delta_{jr} + \epsilon_{ijk}$$

where y are LDL-cholesterol data
i indexes diets
j indexes subjects
k indexes diet repeats for subject j
μ is the overall mean
$\boldsymbol{\beta}$ is the vector of slopes for covariates $\boldsymbol{x}$
τ is the overall diet effect on LDL
γ is the subject effect
λ is the singular value for component r
v is the eigenvalue score for diet i and component r
δ is the eigenvalue score for subject j and component r
ϵ is random error

For the data and model just described, $k = 1$ (i.e., no repeats), γ is considered to be fixed, and there are no covariates.

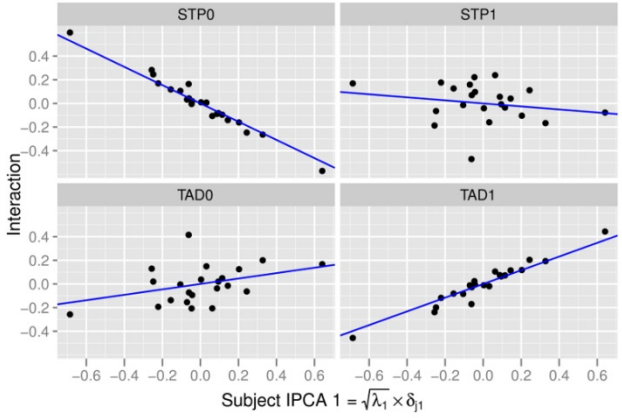

Fig. 2. "Residual" values on the *y* axis, plotted against the rotation and scaling performed by the first principal component, on the *x* axis.

Figure 2 may help to understand in a graphical way what the principal components decomposition is doing. Essentially, the first principal component rotates and scales the residuals in such a way that, at least for the STP0 and TAD1 diets, the residuals can be represented by a line. Calculations for degrees of freedom have been reviewed in (4).

There is software available to perform this analysis using SAS ([10]; SAS macros are available at http://www.k-state.edu/stats/facultypages/ammi_macros.htm) and R (11), package gnm (13). We show code and output from the R software, since R was used for the analysis of the data presented here.

The following code was used to fit the data after the gnm package was installed and loaded and the lp dataset read in.

```
maineffects1 <- gnm(LDLC ~ trt + ID, data=lp)
bilinear1 <- update(maineffects1, . ~. + Mult(trt, lp$ID))
bilinear2 <- update(maineffects1, . ~ . + instances (Mult(trt, lp$ID),
2))
anova(maineffects1,bilinear1,bilinear2,test="F")
```

The first line fits a basic ANOVA model. The second line updates the ANOVA model with a multiplicative interaction term using the first principal component. The third line updates the ANOVA model with the first and second principal components. The fourth line tests whether the increased complexity of the models is an improvement, output on Table I.

Based on this output, both the first and second principal components appear to be necessary.

To examine the relative contributions to the total variance, as sums of squares, from each of the terms in the bilinear2 model (two principal components), ANOVA (bilinear2) was run, with the output displayed on Table II. The columns titled "deviance" give the sums of squares (pseudo sums of squares for multiplicative interaction components and estimated within-subject variation). The largest source of variation is among subjects (ID). For the two sources that relate to treatment, the inconsistent diet effect (interaction, summing over the two principal components) is about 60% as large as the consistent (main) diet effect. Note that the residual sum of squares (data minus main effects) is 3.2190, only 0.2882 of that (9%) is within-subject error.

Table I. Analysis of deviance

Resid.	Df	Resid. Dev	Df	Deviance	F	Pr(>F)
1	63	3.2190				
2	40	1.3372	23	1.8818	5.3934	0.0002225 ***
3	19	0.2882	21	1.0490	3.2930	0.0057560 **

Table II. ANOVA (bilinear2) output

	Df	Deviance	Resid. Df	Resid. Dev
NULL			87	27.9587
trt	3	4.8605	84	23.0983
ID	21	19.8793	63	3.2190
Mult(trt, lp$ID, inst = 1)	23	1.8818	40	1.3372
Mult(trt, lp$ID, inst = 2)	21	1.0490	19	0.2882

Description and Analysis of Dataset 2

In this study, there was replication (i.e., subjects each replicated one of three diets) to directly compare estimates of the subject–diet interaction using traditional ANOVA versus AMMI. Since the two methods do not necessarily capture the same effect, we wanted to verify that they yield similar estimates for these kinds of data.

The 16 subjects were randomly assigned to a treatment sequence consisting of three amounts of pistachios fed in a controlled diet for three weeks each: 0 ounces (control), 1.5 ounces, and 3 ounces of pistachios per day. Each subject participated in two different diets, with one diet repeated twice (one subject did not repeat a diet).

This is an incomplete block design experiment with each subject a block. The missing cells in the matrix of subjects by treatments prevent performing a singular value decomposition. Instead, we did three analyses based on two-diet subsets (so the matrices had no missing values). The variance estimates for the interaction terms calculated with both methods are given in Table III. While variance estimates for the two-diet subsets are similar to each other, they are not identical, likely due to both the small number of subjects in the subsets and the fact that the two-diet subsets are attempting to capture the subject–diet interaction with only one principal component.

From dataset 1 and another published study involving moderate alcohol consumption (1), both using nonreplicated crossover designs, we had 26 dependent variables available. Using AMMI, we found that 19 (73%) had significant subject–diet interactions. This suggests that subject–diet interactions are common in nutrition studies.

Conclusions

Despite the fact that essentially no nutrition studies using crossover designs test for a subject–diet interaction effect, researchers in the field generally understand that not every subject responds to a diet in the same way, as we have formally demonstrated in this paper. It is not clear to us why, when smaller effects, such as period and carryover, are fussed over and estimated, the potentially much more important interaction effect is ignored.

Using a multiplicative decomposition to estimate this interaction term works well in crossover designs (and is commonly employed in agricultural field trials [5]) and does not require that subjects repeat diets. The additional analysis is a small price for such a potentially large benefit; we feel that nutrition researchers (and other researchers using crossover designs) should routinely adopt this methodology in their analyses.

A longer version of this paper is available in Kramer et al. (9).

Table III. Estimates of the subject–diet interaction variance from ANOVA or a multiplicative decomposition of the interaction.

	Subject–diet variance estimate using mixed model	Variance estimate using a multiplicative decomposition	Number of subjects
Control vs. 1 serving	0.00380	0.01049	5
Control vs. 2 servings	0.40976	0.16071	5
2 vs. 1 serving	0.02592	0.02138	6

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