Drug Effects on Blood Chemistry with Beta-Carotene Supplementation

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Abstract

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1 Introduction

The magic of modern medicine is incredible. Various drugs alleviate symptoms or even cure a sickness that may or may not be fatal to a person. However, individual variability is critically ignored by pharmacotherapeutics. Drugs are typically prescribed the "average" or "usual" dosages, but this could set a dangerous precedent. Depending on the patient, the drug may not be effective in some or cause serious toxic effects in others. Not only that, but the resulting effect may also cause misunderstanding of the drug, often misinterpreting the inadequacy of dosage for the inadequacy for the drug itself. On the other hand, frequently prescribing larger amount of drug dosage for a patient can lead to abandonment, as result in building a resistant. In order to achievement maximum effectiveness of the medicine and safety for the patient, the dosage must account for the patient itself, in other words, individualized (Koch-Weser 1975).

Beta-carotene is a pigment found in fungi, plants, and fruits that has been proposed as a potential preventative measure against cancer. Besdies just cancer, it also has been proposed for other ailments such as cardiovascular and eye diseases amongst other supplementations such as vitamin E, vitamin C, and multivitamins. Thus, it is of interest to study the pharmacokinetics of its effects (Christen 2000).

The object of this paper is to study how different dose levels of beta-carotene affect serum beta-carotene over a period of time. Not only are beta-carotene levels measured each time, but we are also interested in on whether there are any effects of beta-carotene supplementation on vitamin E levels. We are interested because both beta-carotene and vitamin E are lipid soluble, so the supplementation may affect vitamin E levels as well.

2 Methods

2.1 Data Description:

The 46 subjects in this double-blind study, in which neither the experimenter nor the subjects know which subject received which treatment (Cherry 2006), are volunteers are randomly assigned a random dose of beta-carotene supplementation (0, 15, 30, 45, or 60 mg per day). The variables taken at each visit are:

- Beta-carotene: the serum beta-carotene levels taken at the time of the visit(μg/mL)
- Vitamin E: serum vitamin E (μg/mL)

The subjects have their beta-carotene and vitamin E levels measured at monthly visits at a maximum of 16 total measurements. The dosing scheme is unique in that:

1. For months 0-3, all patients were untreated, and thus received a placebo.
2. For months 4-9, they are treated with their respective dosage of the supplement
3. For months 10-15, all patients stopped receiving treatment after month 9 and returned for their visits for their measurements

Several other measurements are taken at the very start of the study to use as a baseline:

- Age: The subject's age at the start of the study
- Gender: In this study, it is a binary variable, with 1 indicating the subject is male, and 0 otherwise
- BMI: (Body Mass Index) ratio of the subject's weight divided by height squared (kg/m^2)
- Chol: The subject's serum cholesterol level (mg/dL)

The missing data is somewhat severe because there are some subjects who have missed the entire study by only having the baseline measurements. There are almost more subjects that did not come to the later measurements when the subjects stop receiving treatment. These missing data can cause biases in out models and lead to wrong conclusions.

2.2 Scientific Questions:

The exact scientific questions that the study will explore is:

1. Does beta-carotene supplementation impact serum beta-carotene levels over time, and if so, is the impact dose-dependent?

   -This question asks if the supplementation affects beta-carotene levels, and whether that effect is different depending on the dosage of the supplement

2. If there are changes to serum beta-carotene levels due to supplementation, are there differences by dose in the rate at which patients return to baseline after supplementation has stopped?

   -This question asks if the rate at which serum beta-carotene levels return to baseline levels after patients stop receiving the supplements, so this question particularly focuses on months 10-15.

3. Quantify whether the effect of beta-carotene supplementation on serum beta-carotene levels over time differs by age, gender, BMI, or cholesterol.

   -This question asks if the supplementation have different changes in beta-carotene levels for different patients for different factors

2.3 Statistical Methods:

2.3.1 Exploring the data:

The objective of the experiment is to study the changes in serum beta-carotene levels over time and how it differs between dosages. A basic spaghetti plot is used to graph the serum beta-carotene levels over time for each individual, with colors differentiating the dosages. A spaghetti plot is crucial in such data because it is vital for longitudinal data since the goal is to see the changes over time. Quantitative variables in a table are

Covariates	Mean (SD)	Column1	Column2	Column3	Column4
	Dose = 0	Does = 15	Dose = 30	Dose = 45	Dose = 60
Number of Patients	35	40	37	32	39
Beta-Carotene	262.43 (133.81)	215.18 (138.19)	223.46 (93.57)	228.32 (114.94)	217.21 (130.44)
Vitamin E	7.97 (1.37)	7.73 (1.34)	7.94 (1.74)	8.19 (0.99)	8.19 (1.95)
Age	56.06 (3.91)	56.30 (4.46)	57.46 (4.00)	55.88 (2.98)	56.62 (5.02)
Gender (Male = 1) (%)	19 (54.3)	20 (50.0)	13 (35.1)	16 (50.0)	19 (48.7)
BMI	26.22 (3.47)	25.69 (3.44)	25.80 (2.52)	25.35 (3.16)	24.95 (2.37)
Cholesterol	214.09 (23.64)	223.00 (28.56)	216.30 (35.12)	213.31 (31.87)	238.44 (37.76)

Table 1 The summary statistics for the covariates from months 0-3, the time before the subjects received their treatment.

Covariates	Mean (SD)	Column1	Column2	Column3	Column4
	Dose = 0	Does = 15	Dose = 30	Dose = 45	Dose = 60
Number of Patients	50	56	61	47	60
Beta-Carotene	227.37 (162.92)	871.93 (591.86)	1,054.75 (640.53)	1,285.08 (740.42)	1,337.33 (738.12)
Vitamin E	7.74 (1.60)	5.86 (1.56)	6.21 (2.14)	5.98 (1.26)	6.56 (1.69)
Age	56.56 (4.05)	56.50 (4.40)	57.46 (4.04)	56.04 (3.21)	55.73 (4.34)
Gender (Male = 1) (%)	33 (66.0)	35 (62.5)	20 (32.8)	25 (53.2)	28 (46.7)
BMI	25.68 (3.36)	26.19 (3.34)	25.85 (2.56)	24.70 (2.94)	24.29 (1.81)
Cholesterol	219.26 (27.93)	225.62 (30.13)	215.00 (33.83)	210.82 (32.05)	231.40 (31.94)

Table 3 The summary statistics for the covariates from months 4-9, the duration of time that the subjects received their monthly treatment.

Covariates	Mean (SD)	Column1	Column2	Column3	Column4
	Dose = 0	Does = 15	Dose = 30	Dose = 45	Dose = 60
Number of Patients	59	65	63	56	63
Beta-Carotene	375.62 (406.55)	1,018.03 (528.45)	1,168.25 (497.75)	1,123.62 (465.79)	1,301.29 (529.19)
Vitamin E	8.26 (1.28)	8.38 (1.16)	8.74 (1.38)	8.58 (1.03)	9.00 (1.05)
Age	56.25 (3.91)	56.57 (4.38)	57.44 (4.04)	55.88 (2.96)	55.76 (4.44)
Gender (Male = 1) (%)	35 (59.3)	35 (53.8)	21 (33.3)	28 (50.0)	28 (44.4)
BMI	25.94 (3.38)	25.71 (3.49)	25.83 (2.53)	25.35 (3.13)	24.57 (2.08)
Cholesterol	216.85 (26.40)	224.57 (28.66)	214.44 (33.53)	213.31 (31.65)	231.96 (32.67)

Table 2 The summary statistics for covariates from months 10-15, the time after the treatment has stopped for all subjects to study how serum beta-carotene levels drop to baseline.

displayed with a mean and standard deviation whereas categorical variables list the number of times the observation has occurred and the percent number overall.

Question 1: Does beta-carotene supplementation impact serum beta-carotene levels over time, and if so, is the impact dose-dependent?

The nature of the question asks if beta-carotene level changes is dose dependent. It is safe to assume that the subjects are independent of each other, i.e., each subject does not affect the outcome of another. Also, the question seems to ask about the overall population, rather one specific subject or group. Thus, we can employ a GEE (Generalized Estimating Equations) model because the data is unbalanced, the outcome is continuous, and each subject has more than one observation. Because of these constraints, the flexibility of GEE allows for ease of fitting a model. I have employed a linear spline with four knots to fit the mean. In this case, the marginal model I have fitted is:

$$E(bcarot_i) = X_i\beta$$

where X_i is the design matrix for the all of the covariates in the linear spline model, and β is the vector with the coefficients for each covariate.

Variable Selection:

This question asks if beta-carotene level changes is dose dependent, thus, I advocate that both the month and dose variable are needed, month, because it is the time variable, and dose, since the question depends on this variable. The other variables are not needed since they are not mentioned in the question, so we leave those out.

Question 2: If there are changes to serum beta-carotene levels due to supplementation, are there differences by dose in the rate at which patients return to baseline after supplementation has stopped?

This question only pertains to after supplementation stopped for the subjects, so we can just model months 10-15. Like question 1, this question seems to ask about the population in general and the subjects are the same, so they are presumably independent as well, so I employ a GEE model due to its flexibility. Although the dataset is smaller than the original, a linear spline will be employed as well since it makes for ease of interpretation and ease of modeling. The model is similar to in Question 1 since it is a GEE model:

$$E(bcarot_i) = X_i\beta$$

But the X_i and β are similar to their counterparts in Question 1, albeit a bit simpler since the linear spline in this question is much less complex compared to Question 1.

Question 3: Quantify whether the effect of beta-carotene supplementation on serum beta-carotene levels over time differs by age, gender, BMI, or cholesterol.

This question asks if the effect of the supplementation is affected by the other factors, such as age, gender, BMI, or cholesterol. A relatively simple question compared to the other two questions. Only a simple linear regression model is needed. Because the month variable does not enter the question, we are not dealing with longitudinal data, so a simple linear regression is sufficient.

3 Results

3.1 Exploratory Data Analysis:

The summary statistics tables from Tables 1-3 show the mean and standard deviation for continuous variables and the number and percentage of the observations. As expected, the beta-carotene levels in months 0-3 are the lowest amongst the three tables, because it is before the subjects took beta-carotene supplement. Then there is a big jump from months 0-3 to months 4-9 after the treatment, as expected. Months 10-15 have similar mean to beta-carotene levels in months 4-9. This is slightly misleading because there is a significant drop in the levels from month 10 to month 15. Below more accurately portrays the change of beta-carotene over time since it physically shows the change in the levels over the months of treatment and visits.

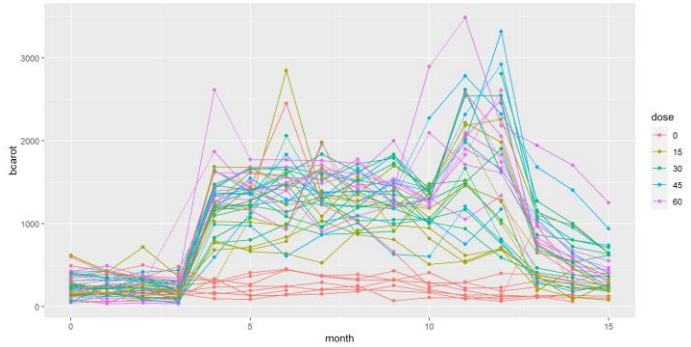


Table 4 Spaghetti plot of beta-carotene level for each subject over time

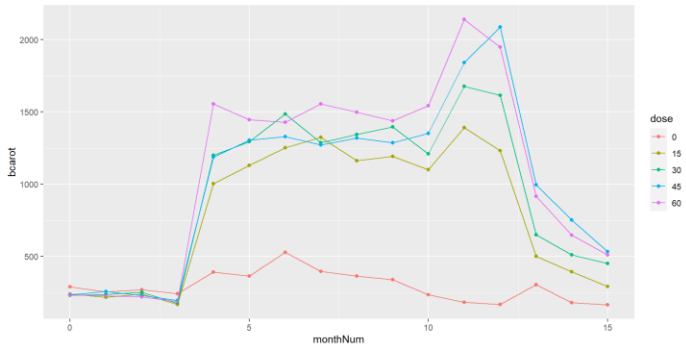


Table 5 Graph of mean serum beta-carotene levels over time grouped by dosages.

Vitamin E levels are also measured at every visit, but there does not seem to be any significant changes from Tables 1 and 2. However, there is a somewhat significant rise in Vitamin E levels in months 10-15, suggesting that stopping the treatment after having high levels of beta-carotene seem to trigger a rise in Vitamin E level, though it is not concrete.

Question 1:

After fitting the GEE model, the model looks like:

$$E(bcarot_{ij}) = \beta_0 + \beta_1 Month + \beta_2 (Month - 3)_+ + \beta_3 (Month - 4)_+ + \beta_4 (Month - 10)_+ + \beta_5 (Month - 12)_+ + \beta_6 1(Dose = 15) Month + \beta_7 1(Dose = 30) Month + \beta_8 1(Dose = 45) Month + \beta_9 1(Dose = 60) Month + \beta_{10} 1(Dose = 15)(Month - 3)_+ + \beta_{11} 1(Dose = 30)(Month - 3)_+ + \beta_{12} 1(Dose = 45)(Month - 3)_+ + \beta_{13} 1(Dose = 60)(Month - 3)_+ + \beta_{14} 1(Dose = 60)(Month - 3)_+ + \beta_{15} 1(Dose = 15)(Month - 4)_+ + \beta_{16} 1(Dose = 30)(Month - 4)_+ + \beta_{17} 1(Dose = 45)(Month - 4)_+ + \beta_{18} 1(Dose = 60)(Month - 4)_+ + \beta_{19} 1(Dose = 15)(Month - 10)_+ + \beta_{20} 1(Dose = 30)(Month - 10)_+ + \beta_{21} 1(Dose = 45)(Month - 10)_+ + \beta_{22} 1(Dose = 60)(Month - 10)_+ + \beta_{23} 1(Dose = 15)(Month - 12)_+ + \beta_{24} 1(Dose = 30)(Month - 12)_+ + \beta_{25} 1(Dose = 45)(Month - 12)_+ +$$

$\beta_{26}1(Dose = 60)(Month - 12)_+ + \beta_{27}1(Dose = 15) + \beta_{28}1(Dose = 30) +$
$+\beta_{29}1(Dose = 45) + \beta_{230}1(Dose = 60)$

Though the model looks very complicated, the model itself is not super complicated because it is merely a linear spline model. However, because this is a GEE model, the likelihood ratio test cannot be conducted since there is no distribution assumption. Because of that fact, a different model selection technique must be used, the QIC (Quasilikelihood under the independence model criterion). Comparing several other models, this model gives the best combination of low QIC and significant covariates. Below gives the ANOVA analysis of the covariates, which provides analysis on the significance of a covariate on a population level. Assuming a $\alpha = 0.05$ test, we can be 95% confident that the dose covariate is significant in our model. This means that we are 95% confident that the mean serum beta-carotene levels is dose dependent.

```
Analysis of 'Wald statistic' Table
Model: gaussian, link: identity
Response: bcarot
Terms added sequentially (first to last)

                    Df    X2 P(>|Chi|)
dose                 4  87.6   < 2e-16 ***
month                1  52.5   4.3e-13 ***
spline_month3        1 130.9   < 2e-16 ***
spline_month4        1 168.6   < 2e-16 ***
spline_month10       1 103.8   < 2e-16 ***
spline_month12       1  33.5   7.1e-09 ***
dose:month           4  73.8   3.6e-15 ***
dose:spline_month3   4  22.3   0.00017 ***
dose:spline_month4   4  41.4   2.3e-08 ***
dose:spline_month10  4  93.7   < 2e-16 ***
dose:spline_month12  4  61.6   1.3e-12 ***
---
```

Figure 1 ANOVA test for the model, every covariate, including dose, is significant.

Question 2:

Similar to Question 1, after fitting the GEE model, the equation should look like:

$$E(bcarot_{ij}) = \beta_0 + \beta_1 1(Dose = 15) + \beta_2 1(Dose = 30) + \beta_3 1(Dose = 45) + \beta_4 1(Dose = 60) + \beta_5 Month + \beta_6 (Month - 11)_+ + \beta_7 (Month - 13)_+ + \beta_8 1(Dose = 15) Month + \beta_9 1(Dose = 30) Month + \beta_{10} 1(Dose = 45) Month + \beta_{11} 1(Dose = 60) Month + \beta_{12} 1(Dose = 15)(Month - 11)_+ + \beta_{13} 1(Dose = 30)(Month - 11)_+ + \beta_{14} 1(Dose = 45)(Month - 11)_+ + \beta_{15} 1(Dose = 60)(Month - 11)_+ + \beta_{16} 1(Dose = 15)(Month - 13)_+ + \beta_{16} 1(Dose = 30)(Month - 13)_+ + \beta_{16} 1(Dose = 45)(Month - 13)_+ + \beta_{16} 1(Dose = 60)(Month - 13)_+$$

Once again, the equation looks very complex but the model at the end is simple. The complexity comes from the interaction term, just like in the model in Question 1. However, the question asks if the rate at which each dosage falls to baseline is the same, so it is best to compare the slopes. From testing the hypothesis and letting all slopes equal to 0, the slope for dosage of 15 fails to reject the null hypothesis at the $\alpha = 0.05$, so there is not sufficient evidence to prove the claim that the slope is different from the others. We cannot conclude that the rates at which serum beta-carotene return to baseline is different by dosage. The 95% confidence of slope estimate ranges from -3616 to 696, so it captures 0 in the interval, which shows that it is not significant.

Question 3:

Question 3 asks whether age, gender, bmi, or cholesterol levels affect the mean serum beta-carotene levels over time. The question is not constrained by dosage, so a simple linear regression can be used to fit the model. In order to see if any of those covariates affect the mean, it is imperative to observe the interaction term between the time variable (month) and all the mentioned variables. For the interaction between age and month, the 95% confidence interval for the coefficient is (-0.0184, 1.2804). For gender and month, the confidence interval is (-93.735, 0.135). For bmi and month, the confidence interval is (0.028, 10.863). For cholesterol, the confidence interval is (-0.00214, 0.39). All these intervals capture 0 within their respective intervals for the interactive term. There is not sufficient evidence to prove the claim that mean serum beta-carotene levels differ by age, gender, BMI, and cholesterol.

```
Call:
lm(formula = bcarot ~ month * (age + male + bmi + chol), data = Dose3_9)

Residuals:
    Min      1Q  Median      3Q     Max
-1284.8  -419.8    48.3   355.9  1791.5

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  2.30e+03   1.85e+03    1.24     0.22
month       -2.43e+01   2.98e+02   -0.08     0.94
age         -1.25e+01   2.39e+01   -0.52     0.60
male         4.16e+01   1.96e+02    0.21     0.83
bmi         -5.09e+01   3.40e+01   -1.50     0.14
chol        -5.07e-03   2.92e+00    0.00     1.00
month:age    6.31e-01   3.82e+00    0.17     0.87
month:male  -4.68e+01   3.15e+01   -1.49     0.14
month:bmi    3.31e+00   5.47e+00    0.60     0.55
month:chol   1.94e-01   4.67e-01    0.42     0.68
```

Figure 2 Esimate and standard error for each covariate in the model.

4 Discussion

4.1 Conclusion:

This experiment studies the effect of dosage of serum beta-carotene and examine how it changes over time. The experiment cleverly separates the time periods into three periods, first being the control where no subjects receive treatment, the second where subject gets their treatment with their randomized dosages, and third, where the subject stops receiving treatment so that the rate at which the serum beta-carotene levels return to baseline can be observed.

Question 1 asks if the dosage impacts the mean serum beta-carotene levels over time, and it is conclusive that at a 95% level, dose impacts the mean. A GEE model is fit with a linear spline trend at various points. Through analysis of variance, it is clear that dose is one of the significant variables at the 95% level, thus making it significant.

Question 2 asks if the rates at which serum beta-carotene levels return to baseline are the same. Through some hypothesis testing by comparing the slopes at which they decrease, at least one of the doses had a slope that is captured by 0 in its 95% confidence interval, thus it can not be concluded that the rate is different by dosage.

Question 3 asks if any other covariates, namely age, gender, bmi, and cholesterol levels, impact the mean of serum beta-carotene levels over time. By directly observing the interavtion term between the other covariates and month, it is easy to see that all 95% confidence interval for the interaction term between month and the respective other covariates captures 0, meaning that none of the covraites have an impact on the mean serum beta-carotene levels over time.

4.2 Limitations:

While it was nice that many of the subjects had their measurements taken at the same time, that tapered off towards the end of the study as more and more subjects did not return for the measurements. This may particularly hit Question 2 hard, since many of the subjects stop at the beginning or middle of the third phase, which the period in which subjects had stop receiving their treatment. This can lead towards a huge bias in Question 2. Another limitation is that, while few, there is some missing data at the beginning of the study, notable the first period where no subjects have received their treatment. While, this may not be super impactful, it can still lead to slight bias in the models due to the missing data (Howe et al 2015).

A Appendix

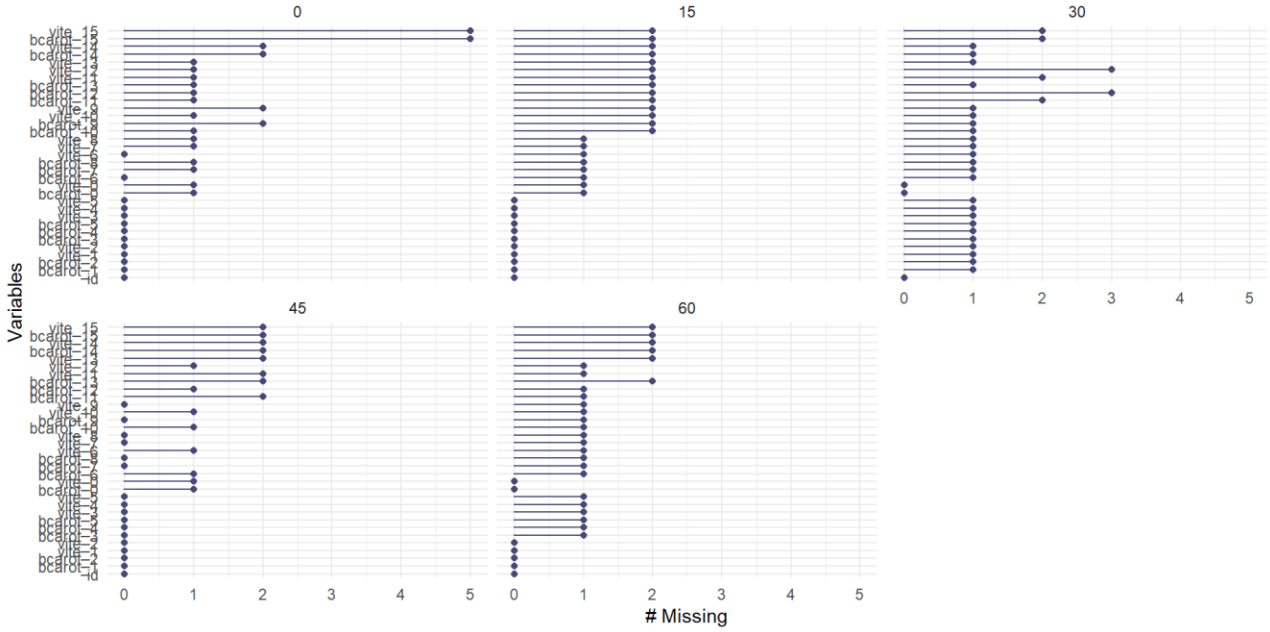
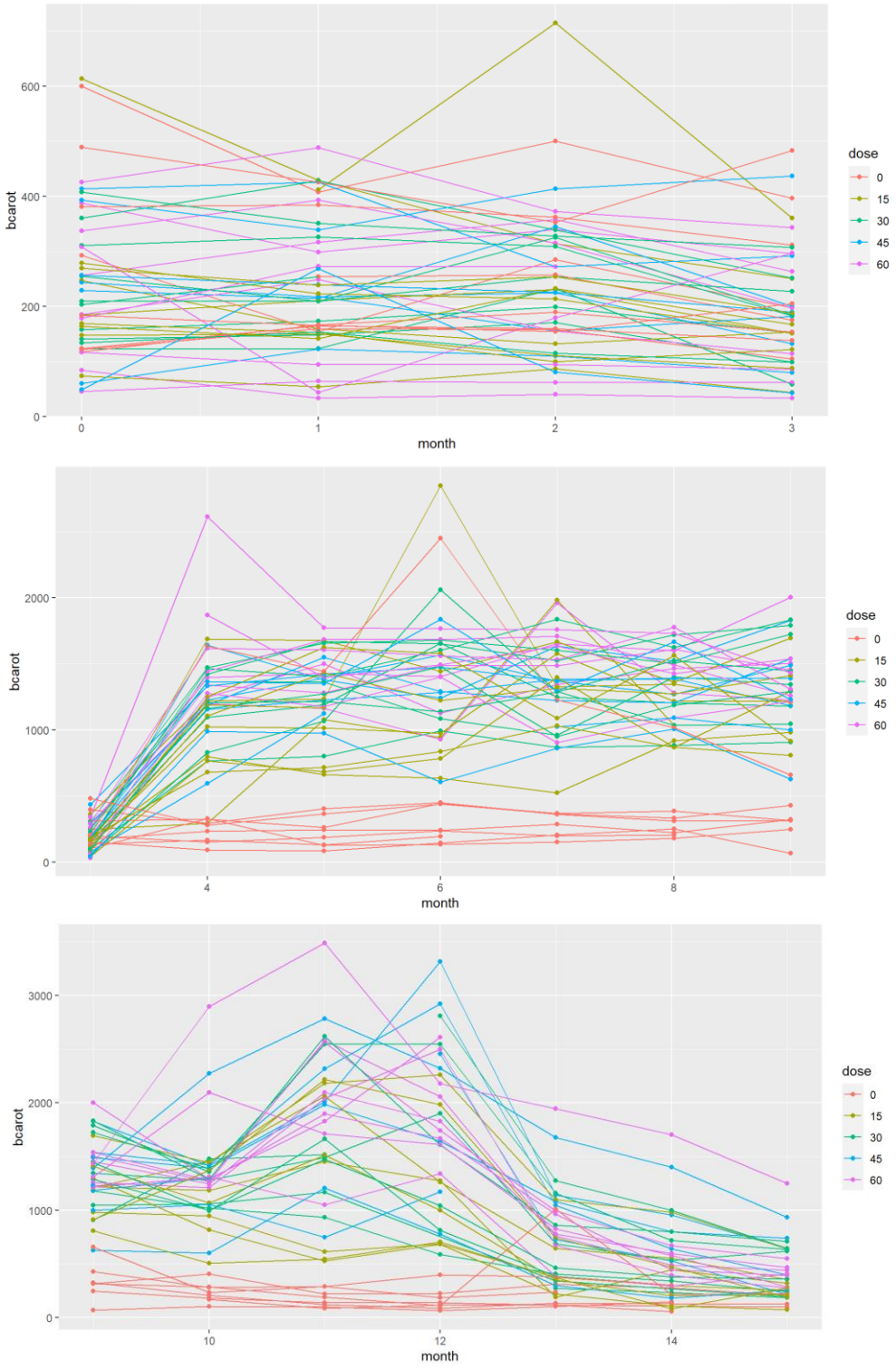


Figure 3 Missing beta-carotene and Vitamin E data, stratified by dose.

B Appendix

Figure 4 Below are the spaghetti plots for the three periods in which the study is separate, from top to bottom, months 0-3, 4-9, 10-15.



C Appendix

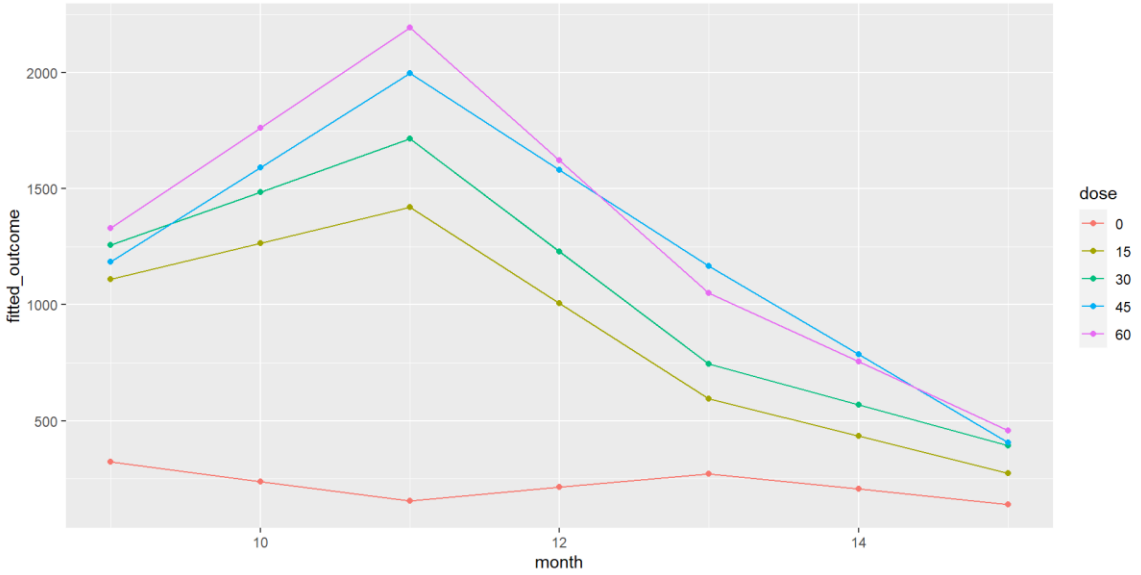


Figure 5 Predicted mean serum beta-carotene levels with linear spline trend in GEE model

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