Modeling the Pharmacokinetics of Beta-Carotene Supplementation: A Longitudinal Mixed Effects Analysis

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Abstract

This study investigates the pharmacokinetics of beta-carotene supplementation in a randomized clinical trial, with a focus on serum concentration levels over time under varying dose levels. Using longitudinal data from 90 participants followed across 16 months, we applied a series of linear mixed-effects models to address five scientific questions related to treatment response, time trends, post-supplementation decay, covariate interactions, and counterfactual predictions. Our models incorporated fixed effects for time, dose, and demographic covariates, along with subject-specific random intercepts and slopes. Quadratic terms and splines were employed to flexibly capture non-linear dynamics. Results indicate significant heterogeneity in serum responses by dose and time, particularly during and after the treatment phase. Prediction-based modeling revealed that higher-dose patients were more likely to maintain elevated beta-carotene levels post-treatment.

Contents

1 Introduction

Beta-carotene is a lipid-soluble carotenoid found in fruits and vegetables and is a well-known precursor to vitamin A, a nutrient essential for vision, immune function, and cellular growth[1]. Due to its antioxidant properties[2], beta-carotene has been investigated for its potential role in preventing chronic diseases, including certain cancers and cardiovascular conditions[3]. Therefore we want to understand its clinical benefits, but before conducting large-scale trials on clinical outcomes, it is essential to understand the pharmacokinetics of beta-carotene and how it accumulates in the serum during supplementation and how it clears once supplementation ends.

To address these questions, a Phase II randomized, double-blind clinical trial was conducted in which healthy volunteers were assigned to one of five beta-carotene dosage levels. Participants were observed over a 16-month period: four months of placebo-only baseline, six months of active supplementation, and six months of post-treatment follow-up. Serum levels of beta-carotene and vitamin E were measured monthly. In addition, covariates such as age, sex, BMI, and cholesterol were recorded at baseline to assess potential effect modification.

In our study we aim to answer these questions:

1. Is supplementation of beta-carotene associated with an increased time-average of serum beta-carotene levels? If so, is the effect of supplementation dose-dependent?
2. Does beta-carotene supplementation impact serum beta-carotene levels over time, and if so, is the impact dose-dependent?
3. 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 is stopped?
4. Quantify whether the effect of beta-carotene supplementation on serum beta-carotene levels over time differs by age, gender, body mass index (BMI), or cholesterol.
5. For two randomly sampled patients at each dose level, provide predictions of their serum beta-carotene levels if they were to have stayed on treatment for an additional 3 months.

2 Data Description

The data we have includes 46 participants that were randomly assigned to receive one of five daily doses of beta-carotene: 0 (placebo), 15, 30, 45, or 60 mg/day. The sampling method involved stratified random assignment to dose groups while be followed over 16 months. The measurements were collected monthly and in those 16 months we had four months during the pre-treatment phase (Months 0–3), six during active supplementation (Months 4–9), and six during post-treatment follow-up (Months 10–15). The key longitudinal outcomes measured at each visit were:

- `bcarot`: serum beta-carotene concentration (μg/mL)
- `vite`: serum vitamin E concentration (μg/mL)

And additional baseline variables collected at randomization included:

- `age`: subject age in years
- `male`: indicator variable for sex (1 = male, 0 = female)
- `bmi`: body mass index (kg/m^2)
- `chol`: serum cholesterol level (mg/dL)
- `dose`: assigned beta-carotene supplementation group
- `month`: time variable indicating visit month (0–15)

The trial had relatively low missing data with most participants completing the majority of scheduled visits. Observed missingness was most likely due to skipped visits rather than dropout, supporting a missing-at-random (MAR) assumption for longitudinal modeling. We also came across one issue with an observation with a clear typo which we manually fixed. More on missingness can be seen in Appendix 6.1

3 Methods

In answering the 5 scientific questions, we perform various strategies. To assess whether beta-carotene supplementation is associated with increased serum beta-carotene levels and whether the effect is dose-dependent(SQ1), we constructed a new outcome variable defined as the change in time-averaged serum beta-carotene between the pretreatment period (months 0-3) and the treatment period (months 4-9). For each subject, we computed the average serum level in each period and then calculated the difference. To compare treatment versus placebo, we used a two-sample t-test with unequal variance to compare this outcome between the placebo group and the pooled treatment groups. To evaluate dose dependence, we performed a one-way ANOVA across all the non-placebo dose levels. The underlying framework compares group-level means to detect differences in serum beta-carotene levels that are attributable to treatment. In both tests, the parameter(s) of interest are the group mean differences in the time-averaged increase.

To investigate whether beta-carotene supplementation impacts serum beta-carotene over time and whether this effect is dose-dependent(SQ2), we only used data from the treatment period from months 4 through 9 as that is the only period supplementation was given, which is why we centered at Month 4. To evaluate the overall effect of supplementation, a new binary variable was created called supplementation, which was 1 for individuals who received any non-zero dose of beta-carotene and 0 for those who received placebo, allowing us to compare beta-carotene rates between supplemented and unsupplemented individuals. We then fit a Linear Mixed Effects Model(1) with fixed effects for month, supplementation status and the interaction between month and supplementation to see if beta-carotene supplementation impact beta-carotene levels over time, while also including all other co-variates, to adjust for confounding. A random intercept was also included to capture differences in average trends between groups over time, while accounting for subject level variability at baseline.

$$\begin{aligned} \text{bcarot}_{ij} = {} & \beta_0 + \beta_1 \cdot \text{Month}^*_{ij} + \beta_2 \cdot \text{Supplementation}_i + \beta_3 \cdot \text{Age}_i + \beta_4 \cdot \text{BMI}_i \\ & + \beta_5 \cdot \text{Male}_i + \beta_6 \cdot \text{Chol}_i \\ & + \beta_7 \cdot (\text{Month}^*_{ij} \cdot \text{Supplementation}_i) + b_{0i} + \varepsilon_{ij} \end{aligned} \tag{1}$$

where $\text{Month}^*_{ij} = \text{Month}_{ij} - 4$,, and $\text{Supp}_i = 1$ for supplemented individuals and 0 for placebo.

Then to assess the dose dependence, we fit a similar Linear Mixed Effects Model(2) using only subjects who received active supplementation but excluding the placebo group to see if the supplementation differs by dose among those who actually received the supplement. In this model, I treated dose group 15 mg/day as the reference group and had fixed effects include month, dose level (30, 45, 60 mg), and interaction terms between the doses and month. This allowed me to evaluate whether the rate of change in serum beta-carotene levels over time differed by dose, building upon the first model. This model is the following

$$\begin{aligned} \text{bcarot}_{ij} = {} & \beta_0 + \beta_1 \cdot \text{Month}_{ij} + \beta_2 \cdot I(\text{Dose}_i = 30) + \beta_3 \cdot I(\text{Dose}_i = 45) + \beta_4 \cdot I(\text{Dose}_i = 60) \\ & + \beta_5 \cdot \text{Age}_i + \beta_6 \cdot \text{BMI}_i + \beta_7 \cdot \text{Male}_i + \beta_8 \cdot \text{Chol}_i \\ & + \beta_9 \cdot \text{Month}_{ij} \cdot I(\text{Dose}_i = 30) + \beta_{10} \cdot \text{Month}_{ij} \cdot I(\text{Dose}_i = 45) \\ & + \beta_{11} \cdot \text{Month}_{ij} \cdot I(\text{Dose}_i = 60) + b_{0i} + \varepsilon_{ij} \end{aligned} \tag{2}$$

where $\text{Month}^*_{ij} = \text{Month}_{ij} - 4$, and *dose 15 mg/day is treated as the reference group*

To evaluate whether there are differences by dose in the rate at which patients return to baseline serum beta-carotene levels after supplementation ends (SQ3), we focused on the post-treatment period from Months 10 to 15. This allowed us to capture the decay phase following active supplementation. We fit a Linear Mixed Effects Model(3) that included both a linear and quadratic term for time, centered at Month 10 which improved interpretability and reduce multicollinearity. We included a quadratic term for time to account for nonlinear patterns as the return to baseline is unlikely to follow a perfectly linear trajectory which is seen by the spaghetti plot later. This allows the model to capture the curvature in the decay trajectory as well as allow the shape of the post-treatment decay curve to vary by dose group which helps us to find out whether higher doses result in faster or slower nonlinear returns to baseline. The model also included dose group, and interactions between dose and both time terms, allowing us to estimate dose-specific decay curves. Additional covariates were included to adjust for potential confounders, and a

random intercept for subject was included to account for individual-level baseline variation like previously.

$$\begin{aligned}\text{bcarot}_{ij} = \beta_0 &+ \beta_1 \cdot \text{Month}^*_{ij} + \beta_2 \cdot (\text{Month}^*_{ij})^2 + \beta_3 \cdot \mathbb{I}(\text{Dose}_i = 30) + \beta_4 \cdot \mathbb{I}(\text{Dose}_i = 45) \\ &+ \beta_5 \cdot \mathbb{I}(\text{Dose}_i = 60) + \beta_6 \cdot \text{Age}_i + \beta_7 \cdot \text{BMI}_i + \beta_8 \cdot \text{Male}_i + \beta_9 \cdot \text{Chol}_i \\ &+ \beta_{10} \cdot (\text{Month}^*_{ij} \cdot \mathbb{I}(\text{Dose}_i = 30)) + \beta_{11} \cdot (\text{Month}^*_{ij} \cdot \mathbb{I}(\text{Dose}_i = 45)) \\ &+ \beta_{12} \cdot (\text{Month}^*_{ij} \cdot \mathbb{I}(\text{Dose}_i = 60)) \\ &+ \beta_{13} \cdot ((\text{Month}^*_{ij})^2 \cdot \mathbb{I}(\text{Dose}_i = 30)) + \beta_{14} \cdot ((\text{Month}^*_{ij})^2 \cdot \mathbb{I}(\text{Dose}_i = 45)) \\ &+ \beta_{15} \cdot ((\text{Month}^*_{ij})^2 \cdot \mathbb{I}(\text{Dose}_i = 60)) + b_{0i} + \varepsilon_{ij}\end{aligned} \tag{3}$$

where $\text{Month}^*_{ij} = \text{Month}_{ij} - 10$, and *dose 15 mg/day is treated as the reference group*

In order to evaluate whether the effect of beta-carotene supplementation on serum beta-carotene levels over time differs by age, gender, body mass index (BMI), or cholesterol (SQ4), we restricted the analysis to the treatment period (Months 4 through 9), when supplementation was actively given. We fit a Linear Mixed Effects Model(4) that included a three-way interaction between time, supplementation status, and each baseline covariate of interest (age, sex, BMI, and cholesterol). Time was centered at Month 4, the start of treatment and the binary variable supplementation was coded same as in equation (1). Each covariate was included as a main effect, a two-way interaction with supplementation, and a three-way interaction with supplementation and time because it allowed us to to see how the time-varying effect of supplementation depends on individual characteristics.

$$\begin{aligned}\text{bcarot}_{ij} = \beta_0 &+ \beta_1 \cdot \text{Month}^*_{ij} + \beta_2 \cdot \text{Supp}_i + \beta_3 \cdot \text{Age}_i + \beta_4 \cdot \text{Male}_i + \beta_5 \cdot \text{BMI}_i + \beta_6 \cdot \text{Chol}_i \\ &+ \beta_7 \cdot (\text{Month}^*_{ij} \cdot \text{Supp}_i) + \beta_8 \cdot (\text{Month}^*_{ij} \cdot \text{Age}_i) + \beta_9 \cdot (\text{Month}^*_{ij} \cdot \text{Male}_i) \\ &+ \beta_{10} \cdot (\text{Month}^*_{ij} \cdot \text{BMI}_i) + \beta_{11} \cdot (\text{Month}^*_{ij} \cdot \text{Chol}_i) + \beta_{12} \cdot (\text{Supp}_i \cdot \text{Age}_i) \\ &+ \beta_{13} \cdot (\text{Supp}_i \cdot \text{Male}_i) + \beta_{14} \cdot (\text{Supp}_i \cdot \text{BMI}_i) + \beta_{15} \cdot (\text{Supp}_i \cdot \text{Chol}_i) \\ &+ \beta_{16} \cdot (\text{Month}^*_{ij} \cdot \text{Supp}_i \cdot \text{Age}_i) + \beta_{17} \cdot (\text{Month}^*_{ij} \cdot \text{Supp}_i \cdot \text{Male}_i) \\ &+ \beta_{18} \cdot (\text{Month}^*_{ij} \cdot \text{Supp}_i \cdot \text{BMI}_i) + \beta_{19} \cdot (\text{Month}^*_{ij} \cdot \text{Supp}_i \cdot \text{Chol}_i) + b_{0i} + \varepsilon_{ij}\end{aligned} \tag{4}$$

where $\text{Month}^*_{ij} = \text{Month}_{ij} - 4$, and $\text{Supp}_i = 1$ for supplemented individuals and 0 for placebo.

To assess what serum beta-carotene levels might have been had supplementation continued beyond the observed treatment period (SQ5), we fit a Linear Mixed Effects Model(5) using data from Months 0 to 9, excluding the placebo group. This allowed us to generate out-of-sample predictions for Months 10 through 12 under the scenario of continued supplementation. To model individual longitudinal trends, we used linear splines with knots at Months 3 and 4, allowing the time trend to change slope at these points where there is the stoppage of placebo treatment and the introduction of supplementation. We included interactions between spline terms and dose group, allowing the shape of the trajectory to vary flexibly across doses. This structure is especially important as the goal is not just to predict average values, but to simulate how each dose group might behave over unobserved time, including potential saturation or slowing of response. To further enhance prediction accuracy, we included baseline covariates (age, BMI, sex, and cholesterol) as fixed effects to adjust for potnetial confounding. A random intercept and random slope for time were included as we want individual-specific predictions. We conducted a Likelihood Ratio test to see if the random slope was needed, and our result(p=5.487e-06) confirms our need for the random slope in order for predictiion. The full model for prediciton is the following

$$
\begin{aligned}
\text{bcarot}_{ij} = {} & \beta_0 + \beta_1 \cdot \text{Month}_{ij} + \beta_2 \cdot (\text{Month}_{ij} - 3)_+ + \beta_3 \cdot (\text{Month}_{ij} - 4)_+ \\
& + \beta_4 \cdot \text{I}(\text{Dose}_i = 30) + \beta_5 \cdot \text{I}(\text{Dose}_i = 45) + \beta_6 \cdot \text{I}(\text{Dose}_i = 60) \\
& + \beta_7 \cdot \text{Age}_i + \beta_8 \cdot \text{BMI}_i + \beta_9 \cdot \text{Male}_i + \beta_{10} \cdot \text{Chol}_i \\
& + \beta_{11} \cdot \text{Month}_{ij} \cdot \text{I}(\text{Dose}_i = 30) + \beta_{12} \cdot \text{Month}_{ij} \cdot \text{I}(\text{Dose}_i = 45) \\
& + \beta_{13} \cdot \text{Month}_{ij} \cdot \text{I}(\text{Dose}_i = 60) \\
& + \beta_{14} \cdot (\text{Month}_{ij} - 3)_+ \cdot \text{I}(\text{Dose}_i = 30) + \beta_{15} \cdot (\text{Month}_{ij} - 3)_+ \cdot \text{I}(\text{Dose}_i = 45) \\
& + \beta_{16} \cdot (\text{Month}_{ij} - 3)_+ \cdot \text{I}(\text{Dose}_i = 60) \\
& + \beta_{17} \cdot (\text{Month}_{ij} - 4)_+ \cdot \text{I}(\text{Dose}_i = 30) + \beta_{18} \cdot (\text{Month}_{ij} - 4)_+ \cdot \text{I}(\text{Dose}_i = 45) \\
& + \beta_{19} \cdot (\text{Month}_{ij} - 4)_+ \cdot \text{I}(\text{Dose}_i = 60) + b_{0i} + b_{1i} \cdot \text{Month}_{ij} + \varepsilon_{ij}
\end{aligned} \tag{5}
$$

where $(\text{Month}_{ij} - 3)_+ = \max(\text{Month}_{ij} - 3, 0)$ and $(\text{Month}_{ij} - 4)_+ = \max(\text{Month}_{ij} - 4, 0)$.

4 Results

4.1 Descriptive Statistics

Table 1 summarizes baseline characteristics across the five dose groups (0, 15, 30, 45, and 60 mg/day) in terms of age, sex, BMI, cholesterol, baseline beta-carotene, and vitamin E levels. The number of participants per group ranged from 8 to 10, with relatively balanced distributions across doses when ensures reasonable comparability among groups. Baseline characteristics were generally balanced across dose groups. Mean age ranged from 55.88 to 57.44 years, and sex distribution was approximately even. BMI values were similar across groups (24.94–26.18 kg/m^2), with the lowest in the 60 mg group (24.94). Baseline cholesterol levels also showed minimal variation (213.31–223.00 mg/dL), indicating comparable lipid profiles. These patterns suggest that randomization was effective in balancing key covariates and minimizing confounding at baseline.

Dose Group	**Placebo (0)**	**Dose 15**	**Dose 30**	**Dose 45**	**Dose 60**
n	9	10	9	8	10
Age (mean (SD))	56.11 (4.04)	56.30 (4.64)	57.44 (4.25)	55.88 (3.14)	56.50 (5.21)
Sex (%)					
Female	4 (44.4)	5 (50.0)	6 (66.7)	4 (50.0)	5 (50.0)
Male	5 (55.6)	5 (50.0)	3 (33.3)	4 (50.0)	5 (50.0)
BMI (mean (SD))	26.18 (3.59)	25.69 (3.58)	25.83 (2.66)	25.35 (3.32)	24.94 (2.43)
Chol (mean (SD))	216.00 (27.17)	223.00 (29.72)	214.44 (35.28)	213.31 (33.54)	238.05 (38.88)
bcarot (mean (SD))	279.44 (175.71)	238.89 (155.00)	233.44 (106.12)	235.14 (143.08)	232.80 (130.83)
vite (mean (SD))	7.95 (2.06)	7.87 (1.45)	8.18 (1.72)	8.07 (0.72)	9.05 (1.23)

Table 1: Baseline Characteristics by Dose Group

The spaghetti plot(Figure 1) shows individual serum beta-carotene levels over time, with bold lines indicating the mean response for each dose group. During the pre-treatment period (months 0–3), serum levels remain relatively low and stable across all groups, confirming the absence of supplementation effects during the untreated phase. After supplementation is initiated (months 4–9), their is a sharp rise in serum beta-carotene with the magnitude of increase dependent on the dose group. Subjects receiving higher doses (45 and 60 mg/day) tend to show more pronounced increases, with some individuals exceeding 2000 µg/mL. The placebo group (dose 0) exhibits no meaningful change throughout the study period, however. After treatment ends at month 9, serum levels do not decline immediately as in several dose groups, especially 30, 45, and 60 mg/day, levels appear to increase slightly or plateau around month 10 before beginning to decline, suggesting a nonlinear and possibly delayed response to treatment stoppage. Overall, Figure 1 reinforces potential dose-dependent treatment effects(SQ1, SQ2) and considerable individual variability, highlighting the importance of flexible longitudinal models(SQ3,SQ5) that incorporate random effects and nonlinear time trends.

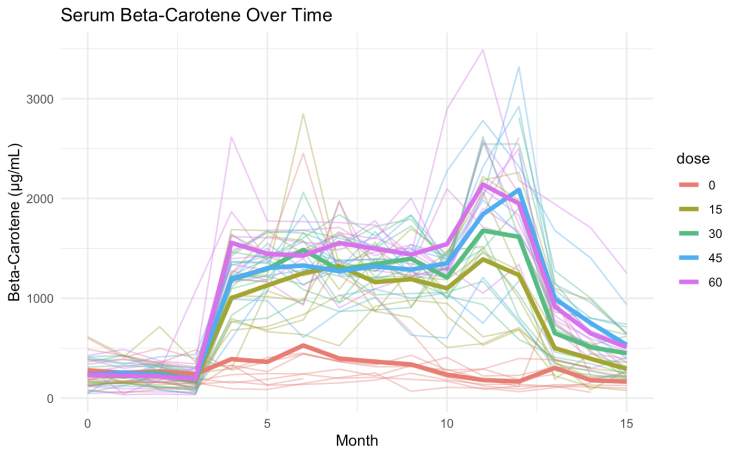

Figure 1: Spaghetti Plot of serum beta-carotene over time by treatment group

4.2 Effect of Supplementation and Dose on Time-Averaged Serum Beta-Carotene Levels

From the results seen in Table 2, the Welch's t-test comparing the change in time-averaged serum beta-carotene levels between the placebo group and the pooled treatment groups showed a statistically significant difference($p < 0.0001$), with a mean increase of 122.31 µg/mL in the placebo group and 1086.91 µg/mL in the treatment group. The one-way ANOVA that was conducted across the four active dose groups (15, 30, 45, and 60 mg/day) also detected a statistically significant difference in mean increases (p = 0.0404), indicating evidence of a dose-dependent effect. Together, these findings provide strong support for the hypothesis that beta-carotene supplementation increases serum levels, and that the magnitude of this effect increases with dose.

Analysis	**Comparison**	**Mean Increase (µg/mL)**	**95% CI**	**p-value**
Welch's t-test	Placebo (122.31) vs Treatment (1086.91)	$\Delta = 964.60$	$[-1291.12, -638.07]$	7.92×10^{-5}
One-way ANOVA	Dose 15, 30, 45, 60	—	—	0.0404

Table 2: Summary of Statistical Tests Assessing Supplementation Effects on Time-Averaged Serum Beta-Carotene

4.3 Longitudinal Effect of Supplementation and Dose on Serum Beta-Carotene Levels

In results from Table 3, we see that based on the model defined in Equation (1), fitted to the treatment period (months 4–9), individuals who received beta-carotene supplementation had significantly higher serum beta-carotene levels compared to those who received placebo. Specifically, supplementation was associated with an average increase of 793.17 µg/mL in serum beta-carotene ($p <$0.001). However, the interaction between month and supplementation was not statistically significant ($p =$0.108), indicating no evidence that the rate of change in serum levels differed between groups over time.

Covariate	**Estimate**	**95% CI**	**P value***
Intercept	1366.98	[-291.71, 3025.67]	0.1136
month*	-19.48	[-59.97, 21.02]	0.3473
supplementation	793.17	[568.64, 1017.69]	< 0.001
age	-5.38	[-25.91, 15.15]	0.6106
bmi	-29.93	[-58.72, -1.15]	0.0483
male	-175.95	[-345.87, -6.03]	0.0420
chol	1.15	[-1.40, 3.69]	0.3839
month*:supplementation	36.89	[-8.92, 82.71]	0.1081

Table 3: Fixed effect estimates with 95% confidence intervals (CI) for equation (1)

To assess dose dependence(Table 4), we used the model defined in Equation (2), restricted to individuals who received active supplementation. No significant interaction terms were found between month and dose group (all $p > 0.1$), suggesting that the rate of change in serum beta-carotene levels over time did not differ substantially across dose levels during the treatment period.

Covariate	Estimate	95% CI	P value*
Intercept	1036.55	[-575.84, 2648.94]	0.1907
month*	35.02	[-3.91, 73.95]	0.0789
dose30	160.47	[-86.00, 406.94]	0.1982
dose45	157.86	[-96.42, 412.14]	0.2166
dose60	378.26	[133.90, 622.63]	0.0033
age	4.42	[-15.57, 24.40]	0.6509
bmi	-18.20	[-47.45, 11.06]	0.2131
male	-157.66	[-319.13, 3.82]	0.0560
chol	1.58	[-0.78, 3.94]	0.1963
month*:dose30	-8.27	[-62.57, 46.03]	0.7668
month*:dose45	-19.89	[-76.14, 36.36]	0.4892
month*:dose60	-43.87	[-98.44, 10.71]	0.1170

Table 4: Fixed effect estimates with 95% confidence intervals (CI) for equation (2)

Overall, we see that beta-carotene supplementation significantly increases serum beta-carotene levels compared to placebo, and while higher doses are associated with higher overall levels, there is no strong evidence that the rate of change in serum levels over time differs either between supplemented and placebo groups or across different dose levels

4.4 Post-Treatment Decline in Serum Beta-Carotene Levels by Dose

Based on the model defined in Equation (3), which focused on the post-treatment period from months 10 to 15, we estimated dose-specific decay trajectories in serum beta-carotene levels using both linear and quadratic time terms centered at month 10. This allowed us to capture non-linear returns to baseline after supplementation ended. The overall time trend showed a negative quadratic effect ($\beta = -31.37$, $p = 0.216$), consistent with a decaying pattern over time, though not statistically significant. Individuals in the 60 mg/day group had higher average serum beta-carotene levels post-treatment compared to the 15 mg/day group ($\beta = 437.62$, $p = 0.093$), suggesting a higher starting point at the beginning of the decay phase, but not a statistically confirmed difference.

To evaluate whether the rate at which participants returned to baseline differed by dose, we examined interactions between dose group and both the linear and quadratic time terms. The interaction between the linear time term and dose 45 was positive ($\beta = 334.18$, $p = 0.090$), suggesting a steeper initial decline compared to 15 mg/day. Most notably, the quadratic interaction for dose 45 was statistically significant ($\beta = -74.64$, $p = 0.050$), indicating a more clear curvature in the decline, consistent with a faster return to baseline. The corresponding interaction terms for doses 30 and 60 were not statistically significant (all $p > 0.1$), implying similar or more gradual decay patterns relative to the lower dose group 15.

Covariate	Estimate	95% CI	P value
Intercept	1766.16	[14.47, 3517.85]	0.2197
month*	-64.87	[-323.38, 193.64]	0.6228
dose30	69.74	[-435.23, 574.72]	0.7873
dose45	202.49	[-331.04, 736.03]	0.4598
dose60	437.62	[-67.83, 943.06]	0.0932
month*_sq	-31.37	[-80.28, 17.54]	0.2163
age	10.96	[-22.70, 44.62]	0.5291
bmi	-35.15	[-88.63, 18.33]	0.2004
male	-233.37	[-533.78, 67.04]	0.1252
chol	-0.22	[-4.37, 3.92]	0.9162
month*:dose30	60.91	[-298.80, 420.63]	0.7404
month*:dose45	334.18	[-49.39, 717.76]	0.0898
month*:dose60	149.22	[-209.83, 508.27]	0.4156
dose30:month*_sq	-15.81	[-85.28, 53.66]	0.6572
dose45:month*_sq	-74.64	[-148.69, -0.60]	0.0500
dose60:month*_sq	-46.07	[-115.26, 23.12]	0.1927

Table 5: Fixed effect estimates with 95% confidence intervals (CI) for Equation(3)

Taken together, these results suggest that the rate of return to baseline beta-carotene levels may differ by dose, with strongest evidence for faster nonlinear decay in the 45 mg/day group. However, there is no clear evidence of dose-dependent differences in the decay trajectory for the 30 mg/day or 60 mg/day groups relative to the reference group.

4.5 Effect Modification by Other Covariates

The results in Table 6 examine whether the effect of beta-carotene supplementation on serum levels over time differs by individual characteristics (SQ4), our linear mixed effects model found that the effect of supplementation varied across covariates, though interaction terms involving time were not statistically significant. Specifically, supplementation had a stronger average effect among younger participants ($supplementation : age : \beta = 78.63, p = 0.034$), with decreasing benefit as age increased. A similar trend was observed for BMI, where the effect of supplementation was somewhat larger for individuals with higher BMI which was close to being significant.

However, none of the three-way interaction terms involving time, supplementation, and any covariate were statistically significant (all $p > 0.1$), indicating no strong evidence that the rate of change in beta-carotene levels over time due to supplementation differed by age, sex, BMI, or cholesterol. Likewise, interaction terms between time and individual covariates (e.g., month × age, month × BMI) and between time and supplementation were not significant, further suggesting that individual characteristics did not meaningfully modify the trajectory of serum beta-carotene levels during treatment.

Covariate	Estimate	95% CI	P value*
Intercept	8207.67	[3013.92, 13401.41]	0.0029 **
month*	-804.60	[-1830.11, 220.91]	0.1250
supplementation	-6747.15	[-12308.65, -1185.65]	0.0203 *
age	-78.07	[-145.07, -11.06]	0.0252 *
male	-356.28	[-814.92, 102.36]	0.1268
bmi	-99.88	[-174.48, -25.28]	0.0110 *
chol	-2.67	[-11.19, 5.85]	0.5415
month*:supplementation	811.33	[-284.29, 1906.96]	0.1483
month*:age	9.44	[-3.60, 22.49]	0.1574
month*:male	75.91	[-37.90, 189.71]	0.1896
month*:bmi	6.54	[-10.50, 23.58]	0.4531
month*:chol	0.16	[-1.52, 1.85]	0.8500
supplementation:age	78.63	[7.12, 150.14]	0.0344 *
supplementation:male	207.23	[-289.09, 703.55]	0.4164
supplementation:bmi	75.17	[-7.10, 157.44]	0.0820 .
supplementation:chol	4.82	[-4.26, 13.90]	0.3025
month*:supplementation:age	-8.92	[-22.88, 5.04]	0.2116
month*:supplementation:male	-80.49	[-201.94, 40.96]	0.1909
month*:supplementation:bmi	-6.04	[-24.57, 12.49]	0.5236
month*:supplementation:chol	-0.30	[-2.09, 1.50]	0.7460

Table 6: Fixed effect estimates with 95% confidence intervals (CI) for Equation (4)

4.6 Predicted Serum Beta-Carotene Levels Under Extended Supplementation

To evaluate expected outcomes under extended supplementation, we generated predictions for Months 10–12 using the spline-based linear mixed model from equation (5). This model was trained on observed data from Months 0 to 9 and incorporated random intercepts and slopes to account for heterogeneity in both baseline levels and patient-specific trajectories.

The fixed effects output revealed that the spline terms for Months 3 and 4 were highly significant, validating the inclusion of flexible time components to capture changes in response during and after the initial supplementation ramp-up. Notably, the interaction between spline terms and dose 60 was statistically significant, suggesting that the highest dose group exhibited different growth or saturation dynamics during treatment(Table 8 Appendix 6.3)

Predicted serum levels for two randomly selected patients per dose group are shown in Table 7. The values indicate sustained or slightly increasing trajectories from Month 10 to 12 for most individuals, especially those receiving higher doses. For example, patients in the 60 mg/day group (IDs 20 and 36) showed continued elevated levels through Month 12, with predicted values exceeding 1400 µg/mL. In contrast, patients in the 15 mg/day group (IDs 11 and 48) exhibited more stabilization which can be seen in Figure 2. From that plot we can see the the continuity between observed and predicted segments for each individual supports the validity of the model and previous things found in other models.

ID	Dose	Age	BMI	Male	Chol	Month 10	Month 11	Month 12
11	15	58	23.06	1	254	847.75	841.57	835.39
48	15	55	26.50	1	201	945.87	953.72	961.56
3	30	56	31.55	1	182	959.55	957.50	955.45
27	30	64	27.95	1	217	1792.96	1858.19	1923.43
12	45	56	23.15	1	216	1316.97	1331.10	1345.23
23	45	60	21.67	0	189	1463.77	1480.69	1497.62
20	60	64	25.67	0	209	1433.28	1418.65	1404.02
36	60	52	24.74	1	223	1539.73	1543.36	1546.98

Table 7: Predicted Beta-Carotene Levels (Months 10–12) for Sampled Patients

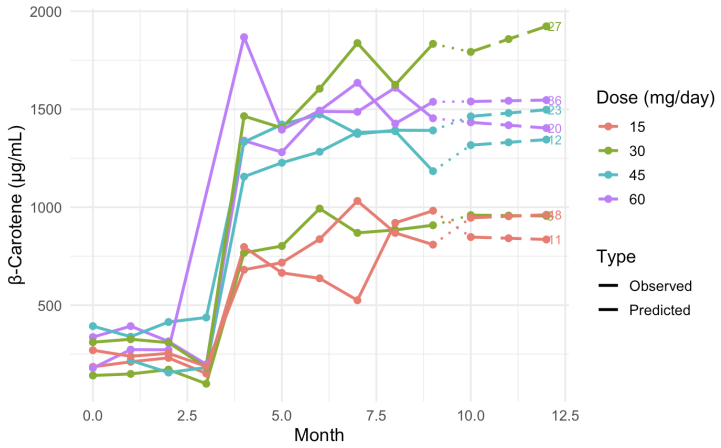

Figure 2: Observed vs. Predicted Beta-Carotene Levels for Sampled Patients

5 Discussion

5.1 Conclusion

Overall, this study aimed to evaluate how different doses of beta-carotene supplementation affect serum beta-carotene levels over time, including post-treatment decay and potential effect modification by individual characteristics. Using a longitudinal dataset from a Phase II trial, our findings consistently show that supplementation leads to significantly higher serum beta-carotene concentrations compared to placebo, with evidence of a dose-dependent effect on average levels. However, we did not observe significant differences in the rate of change during treatment across dose groups, suggesting that while higher doses yield higher levels, the rate over time is similar. Post-treatment analysis revealed nonlinear decay patterns, particularly in the 45 mg/day group, which exhibited a statistically significant quadratic interaction with time. This suggests some dose-specific dynamics in how quickly serum levels return to baseline, although such differences were not consistently observed across all doses. In exploring effect modification, we found that the average effect of supplementation was stronger in younger individuals and those with higher BMI. However, none of the time-dependent interaction terms with individual characteristics were significant, indicating limited evidence that patient characteristics altered the serum beta-carotene trajectory over time. Lastly, predictions under extended supplementation scenarios (Months 10–12) indicated continued elevated or rising levels, especially in higher dose groups. This highlights the potential for prolonged physiological effects beyond the observed treatment window.

In conclusion, beta-carotene supplementation increases serum levels in a dose-dependent manner, with some evidence of differential decay and effect modification. Future studies should investigate long-term accumulation, individual variability, and clinical relevance to better inform dosing strategies.

5.2 Limitations

While the analyses provided valuable insight into the pharmacokinetics of beta-carotene supplementation, we may have several limitations. The sample size within each dose group was relatively small with only 8 to 10 participants per dose where we introduce some uncertainty to that[4]. We also can not rule out residual confounding cannot be ruled out as unmeasured factors may influence serum beta-carotene dynamics and were not captured in this study as we didn't have many variables. Our analyses also assumed that missing data were missing at random (MAR). Lastly, the analysis focused only on serum beta-carotene. Although vitamin E was measured and may interact biologically with beta-carotene, we did not investigate potential joint effects. Future analyses incorporating both biomarkers could help clarify possible nutrient-nutrient interactions[5].

6 Appendix

6.1 Missing Data

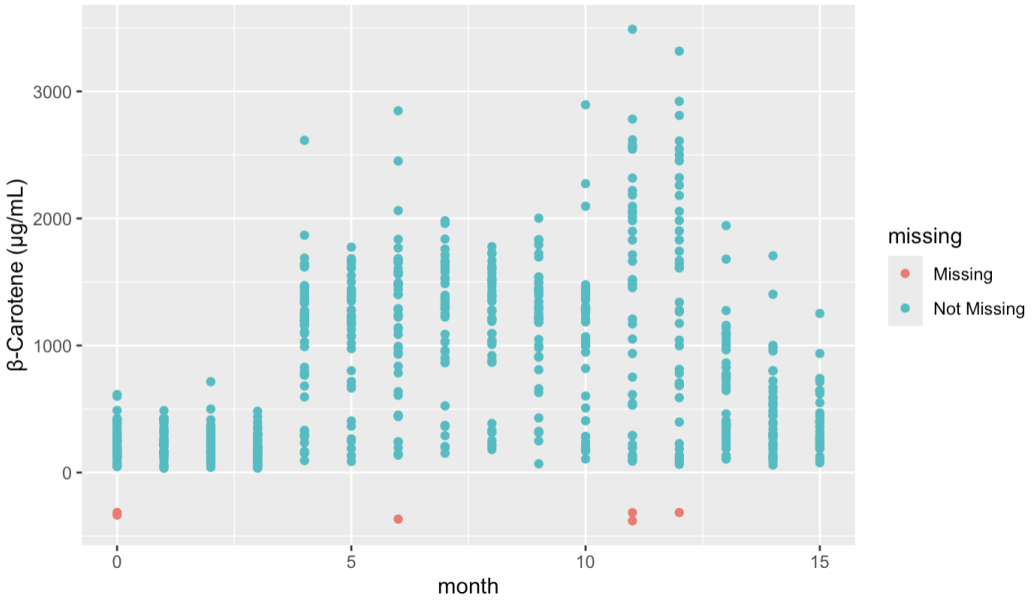

Figure 3: Missing Beta-Carotene Values Over Time

Figure 3 displays the pattern of missingness in serum beta-carotene levels over the 16-month study period. One issue is that there is a single missing value at Month 0. Upon inspection, this was due to a typo involving subject ID 26: the same observation was split across two lines in the raw dataset. This duplicated structure led to one instance appearing as missing. Therfore we manually fixed this and this particular missingness does not reflect a true absence of data.

Aside from that case, the remaining missing values are relatively few and appear to be scattered sporadically across the study months, particularly between Months 11 and 13. These missing values do not follow a systematic pattern across dose groups or individuals, and are not clustered at specific phases of the study, which would suggest drop-out Given this distribution, and the lack of strong time-related or dose-related clustering in missingness, we treat these values as missing at random (MAR). This assumption is appropriate given the clinical trial setting, where occasional missed visits are expected rather than being driven by unobserved outcomes. Therefore, our Linear mixed models, which remain valid under MAR.

6.2 Diagonstics

To assess the adequacy of the linear mixed models used in our analyses, we examined residual diagnostics across all five models. These included quantile-quantile (Q-Q) plots for normality and Pearsons residuals versus fitted value plots for evaluating homoscedasticity and model fit. Overall, the diagnostics reasonably support the validity of the modeling approach. The Q-Q plots for all models show approximate linearity, indicating that the residuals are reasonably normally distributed. While slight deviations were observed in the tails—particularly in models analyzing post-treatment decay and predictions beyond the treatment period—these patterns are minor and not unexpected in longitudinal data. The residuals versus fitted plots generally show no strong evidence of heteroscedasticity or systematic bias, with most residuals scattered symmetrically around zero. The banding patterns observed in some models reflect repeated measures per subject and are consistent with the inclusion of random intercepts. Furthermore, the inclusion of random effects was supported both theoretically and by improved model fit helping to capture subject-level variation and improve predictive accuracy.

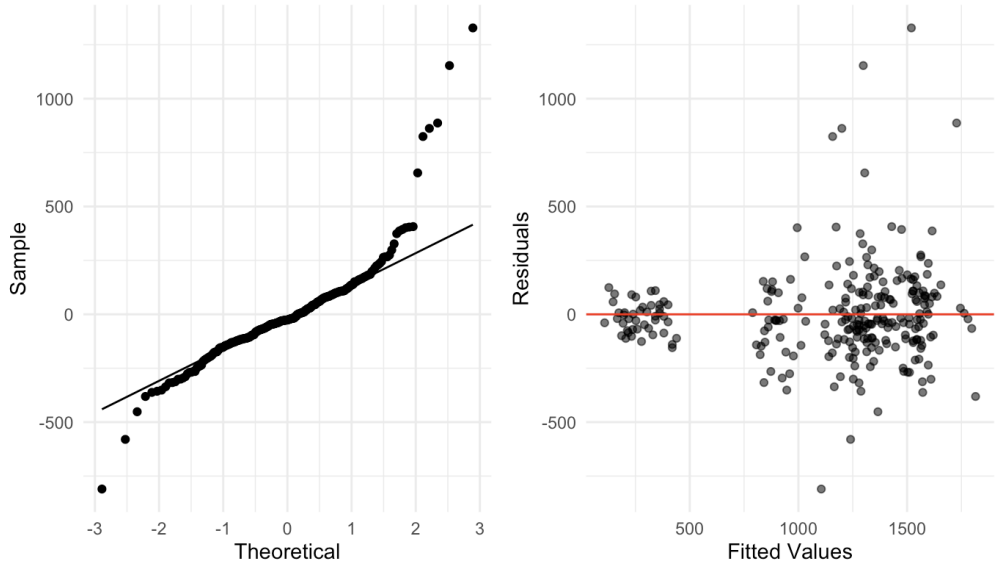

Figure 4: QQ and Pearson plots of the model Equation 1

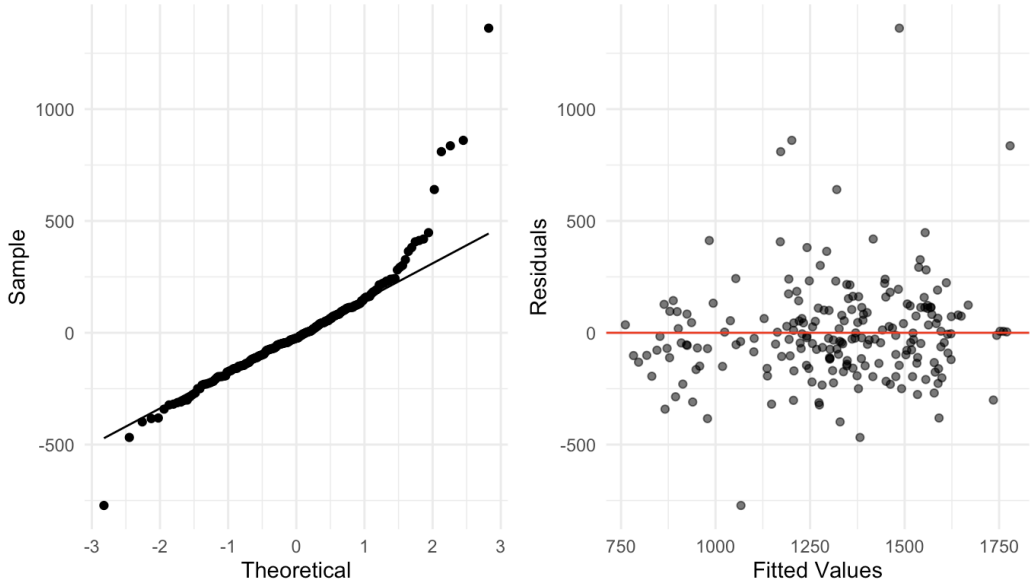

Figure 5: QQ and Pearson plots of the model Equation 2

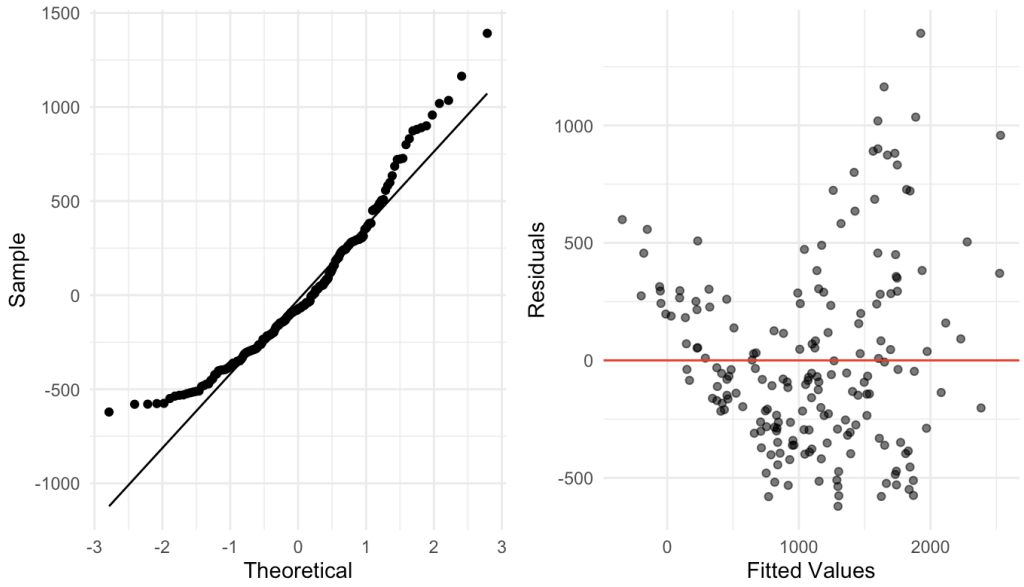

Figure 6: QQ and Pearson plots of the model Equation 3

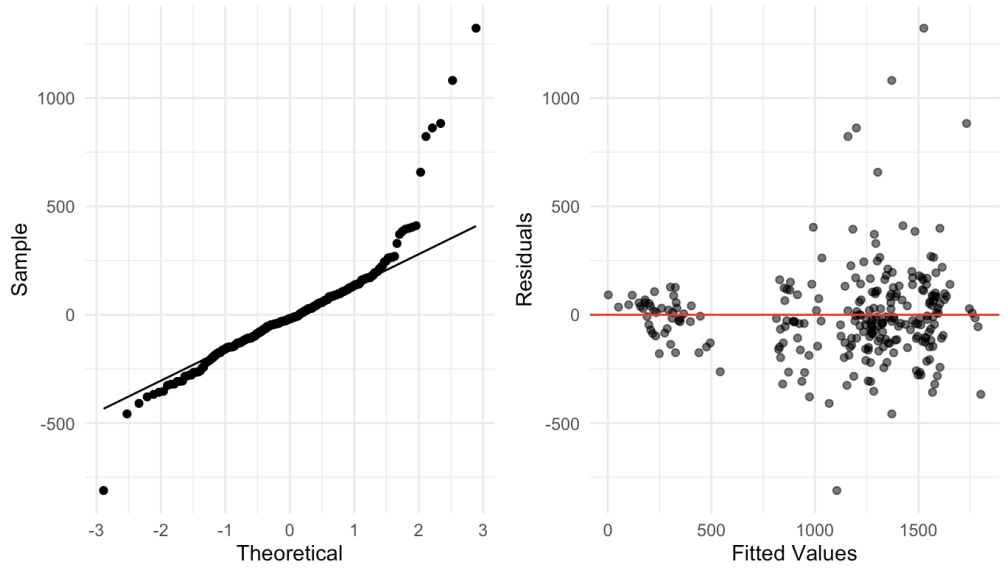

Figure 7: QQ and Pearson plots of the model Equation 4

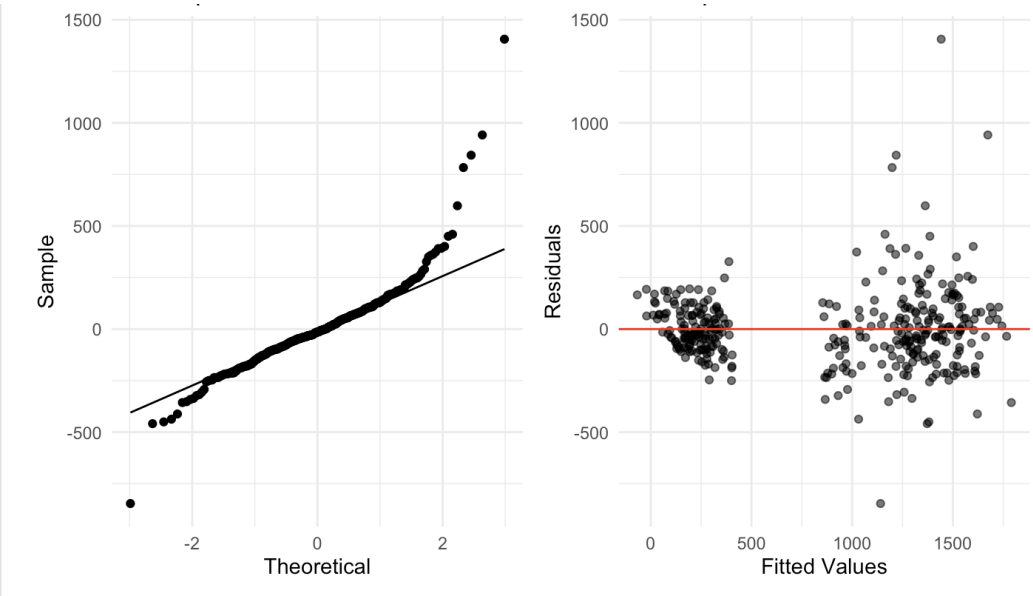

Figure 8: QQ and Pearson plots of the model Equation 5

6.3 Additional Plots or Tables

Table 8: Fixed effect estimates with 95% confidence intervals (CI) for Equation (4)

Covariate	**Estimate**	**95% CI**	**P value**
Intercept	1036.55	[-478.42, 2551.51]	0.1907
month*	35.02	[-3.91, 73.95]	0.0789
dose30	160.47	[-81.10, 402.04]	0.1982
dose45	157.86	[-88.75, 404.47]	0.2166
dose60	378.26	[137.78, 618.74]	0.0033
age	4.42	[-14.48, 23.31]	0.6509
bmi	-18.20	[-46.19, 9.79]	0.2131
male	-157.66	[-312.14, -3.18]	0.0560
chol	1.58	[-0.76, 3.93]	0.1963
month*:dose30	-8.27	[-62.06, 45.51]	0.7668
month*:dose45	-19.89	[-75.56, 35.78]	0.4892
month*:dose60	-43.87	[-98.04, 10.30]	0.1170

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