Dose Analyses for Phase II Trial of Beta-Carotene

Abstract

Background: Beta-carotene is a potent antioxidant that can be taken as a supplement. Studies suggest that supplemental beta-carotene might confer protection against some cancers. It is therefore of great interest to determine optimal doses for safety and effectiveness, and to evaluate any other potential biochemical interactions.

Objective and Methods: The purpose of this study was to analyze the effect of beta-carotene supplementation on plasma levels of beta-carotene and vitamin E. The study was a randomized, double-blind placebo-controlled phase II trial of four different doses of beta-carotene. Forty-six volunteers were randomly assigned to receive one of the four doses of beta-carotene (15, 30, 45, or 60 mg/day) or placebo for 9 months. Data for serum beta-carotene and vitamin E was obtained at baseline, at 3 months post-randomization, and at 9 months post-randomization.

Results: The difference in plasma beta-carotene between baseline and nine months was significantly greater for the treatment groups (doses 15 – 60mg/day) versus the placebo group ($P < 0.001$). The placebo group had a mean decrease of 101.6, while the treatment groups had a mean increase of 1374.1 between baseline and nine months. The increase was 1475.7 greater for the treatment groups (95% confidence intervals 1291.8 to 1659.6). Plasma vitamin E decreased in all groups between baseline and nine months, but was significantly lower for the treatment groups (dose 15 – 60mg/day) versus the placebo group ($P = 0.0061$). The placebo group had a mean decrease of 0.86, while the treatment groups had a mean decrease of 1.85 between baseline and 9 months. The decrease was 0.98 lower for the treatment groups (95% confidence interval 0.32 to 1.64).

Conclusions: Supplementation with beta-serum carotene increases serum beta-carotene levels, but may be associated with a decrease in serum vitamin E levels, especially for higher dose groups.

Comment [A1]: For emphasis, you might say "for all treatment groups combined"

It would also be of interest to at least descriptively provide information about a dose response.

Introduction

Beta-carotene, a carotenoid that is a provitamin form of vitamin A, is a potent antioxidant, and may offer protective benefits against various cancers. Epidemiological studies have shown that cancer risks are inversely correlated with blood retinol and dietary beta-carotene.[1] Low intake of fruits and vegetables and carotenoids have consistently been associated with an increased risk of lung cancer, and low levels of plasma beta-carotene have been associated with subsequent development of lung cancer.[2,3] However, studies have shown large variations among individuals in plasma levels of beta-carotene after ingestion of supplemental beta-carotene.[4] Certain attributes of subjects taking oral beta-carotene supplementation may affect serum response. In a recent study by Costantino et al., subjects with a lower body mass index had greater increases in serum beta-carotene levels.[5] This same study also noted greater increases in serum beta-carotene for those individuals with the highest baseline high density lipoprotein cholesterol levels. Both of these findings may have implications for the optimal dose appropriate for individuals in relation to total body fat and cholesterol. Thus, studies to assess the effect of different doses on plasma beta-carotene levels are warranted.

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In addition to the evaluation of dose on plasma beta-carotene, this study also aimed to assess the effect of beta-carotene on plasma vitamin E levels. Beta-carotene and vitamin E are both lipid-soluble, and increased levels of beta-carotene could potentially affect vitamin E levels. Animal studies have pointed towards a possible antagonistic effect of the lipid-soluble vitamin A on vitamin E status, with excess vitamin A causing a decrease in absorption of vitamin E.[6] However, in a randomized, double-blind trial by Willett et al. on 59 human subjects, a daily beta-carotene supplement of 30 mg did not affect vitamin E

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levels after a period of 16 weeks.[7] An assessment of any potential influence of beta-carotene on vitamin E status is important since low serum vitamin E has been associated with an increased risk of cancer.[3]

Study Objectives:

The main objective of this study was to examine the effect of supplementation of beta-carotene on serum beta-carotene and vitamin E over a prolonged period of time. Our secondary objective was to evaluate the dose-response of beta-carotene supplementation vs. placebo on beta-carotene and vitamin E over the same time period. This information can help to determine the optimal dose for effectiveness, and help refine future studies evaluating the outcomes of long-term beta-carotene supplementation. Secondary analyses evaluated the differential effects of body mass index (BMI), percent body fat, cholesterol and sex on response of beta-carotene and vitamin E levels to beta-carotene supplementation.

Source of Data

Data was collected as part of a randomized, double-blind placebo-controlled phase II trial conducted at one study site. Forty-six volunteers from Seattle, Washington were randomly assigned to receive one of the four doses of beta-carotene (15, 30, 45, or 60 mg/day) or placebo for 9 months. Baseline biometric data of age and gender, as well as measurements of weight, height and percent fat were obtained. Height and weight were converted to body mass index by standard methods. Venous blood samples were obtained at baseline for serum levels of beta-carotene and vitamin E, as well as fasting total cholesterol. Venous blood samples were repeated at three months post-randomization, and nine months post-randomization for beta-carotene and vitamin E. The values for vitamin E and beta-carotene were converted into a value for area under the curve, factoring in time in relation to serum levels. There were no significant patterns of missing data, although a total of five subjects missed follow-up at nine months (randomized to doses = 0, 15, 15, 30 and 45 mg/day), and 1 subject missed follow-up at three and nine months (dose = 60 mg/day).

Statistical Methods

Patient characteristics at baseline (month 0) by dose group are listed in Table 1. The mean, standard deviation, minimum, and maximum are given for continuous variables, and counts are given for sex, the only discrete variable. Table 2 lists mean serum beta-carotene and vitamin E at baseline, after three months, and after nine months of the study. The mean difference in serum beta-carotene from baseline to three months and baseline to nine months is also given; the same is listed for vitamin E.

The aim of the primary analyses was to determine if mean differences in serum beta-carotene from baseline to three months and baseline to nine months differed between dosage groups; the same question also applied to serum vitamin E. Student's t-test was used to compare the mean differences for serum beta-carotene. To evaluate individual dose response vs. no treatment, dose groups 15, 30, 45, and 60 mg/day were compared to the placebo group. To try to identify evidence of a dose response, all treatment dose groups were compared to each other. A final test, checking for a relationship between treatment and non-treatment, was done to compare the mean difference for the placebo group to the mean difference for all subjects in treatment dosage groups. The same tests were repeated for mean differences in serum vitamin E.

Student's t-test was also used to examine the possibility of effect modification by sex and body fat (as measured by BMI). Subjects were dichotomized into two groups by BMI: overweight (BMI > 25) and normal weight (BMI ≤ 25). T-tests were then done to compare mean differences for both beta-carotene and vitamin E between BMI groups for placebo group and all treatment dose groups. T-tests were also done to compare mean differences between men and women for the placebo group and all treatment dose groups.

Comment [A2]: Okay, but even though the documentation did not make this entirely clear, it is most likely the case that the primary interest for beta carotene levels was the dose-response, while for vitamin E your approach was likely a good one.

We will discuss this more next quarter.

Comment [A3]: Actually, it was Tucson, Arizona, but good to note that it was one site

Comment [A4]: They were actually plasma levels.

Comment [A5]: You would likely need to explain how this would factor in time (it is just an average value over time).

Comment [A6]: OK to note here, but as missingness is a post-randomization variable (and hence potentially caused by the treatment), it is good to treat as a Result as well.

Comment [A7]: These are results. You should refer to tables in the Results, not in Stat Methods.

Comment [A8]: You also (very correctly) provide min and max for these post-randomization measurements. This is a very important thing to include, because the treatment could lead to toxic levels (whatever those might be) in just a few subjects.

Comment [A9]: I had said that you did not have to worry too much about multiple comparisons, so this is OK. Next quarter we will try to avoid having so many comparisons.

Comment [A10]: This wording is not clear. That is, from this wording I cannot tell whether you did the right thing or not. And you never gave the results to be able to allow me to make a more educated guess. It was probably not strictly the t test. Instead it would be more akin to a regression analysis—which we will discuss next quarter.

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For all t-tests, subjects missing measurements after three months or nine months were excluded from comparisons of relevant mean differences. Estimated differences of the means, 95% confidence intervals, and one-sided p-values are listed in Table 3. All t-tests used robust standard errors to allow for different variance between dose groups, and no adjustment was made to account for multiple comparisons.

Comment [A11]: Good to note (and the approach I most often use.

Results

Results were calculated with data from a total of 46 subjects consenting to study. One subject in the highest dose beta-carotene arm had no beta-carotene levels and vitamin E levels at three or nine months determined. At nine months there were one subject in the placebo arm, two patients in the 15 mg/day arm, one subject in the 30 mg/day arm, and one subject in the 45 mg/day arm all of whom had no serum beta-carotene level or vitamin E level. There was no statistical difference when comparing numbers of missing patients between beta-carotene dosing groups at three months and nine months.

Comment [A12]: Good to think about (but hardly surprising given the small sample sizes).

Overall, significant differences are present when each individual treatment dose group's mean serum beta-carotene levels is compared to mean placebo levels at both three and nine months (p-value <0.0001). Between each arm of the supplementation groups, evaluation of the primary outcome of serum beta-carotene levels at three and nine months revealed no difference in the serum beta-carotene levels (all p-values >0.05) except when comparing the three-month and nine-month serum beta-carotene levels for 60 mg per day to 15 mg per day (p-value 0.016).

Comment [A13]: Presumably, you have provided these results in a table. You might tell us where.

Mean change in beta-carotene levels at three and nine months were compared to placebo and are also statistically significant for each dosage group (p-values <0.001), and comparison between dosing groups again reveals a statistical difference in between the 15 mg/day and 60 mg/day dosing groups at three months and nine months. The mean change in beta carotene for 15mg/day, 30mg/day, 45mg/day, and 60 mg/day groups compared to the mean change in the placebo groups are all statistically significant (p-values at 3 months all <0.0001 and at 9 months 0.0010, <0.0001, 0.0003, and <0.0001 respectively).

Comment [A14]: You are placing way too much emphasis on statistical significance. First provide the estimates and CI, then comment on the p values. This is especially important in a study with such low sample sizes: You lack precision.

Based on absence of difference in mean changes of serum beta carotene by dose and absence of difference in mean serum beta-carotene levels by dose, further analysis of serum beta-carotene levels is done by presence of treatment versus placebo in the rest of the analysis which increases the sample size under the therapy condition without greatly increasing the variance of serum beta carotene levels measured for the treatment groups.

Comment [A15]: This is an exploratory analysis that is very much prone to inflation of the type I error. Typically I would have done these in reverse order, if I were going to do both of them. And then the test of treatment vs placebo was just to establish that the treatment was doing something. I would not have presumed that there was no dose effect. I would then have descriptively talked about the dose response, whether I had sufficient precision to detect it or not.

We will talk further about thiis next quarter.

Evaluation of the secondary outcome of serum vitamin E levels at three and nine months reveals no statistically significant differences between placebo and each dosage group at randomization (p-values >0.05). At three months, there is a significant increase in serum vitamin E levels when compared to placebo group serum vitamin E at randomization (p-values 0.0706, 0.0053, 0.0019, 0.0102, and 0.0286 for placebo, 15mg/day, 30mg/day, 45mg/day, and 60mg/day groups respectively), but no statistical significance when comparing the mean differences between treatment groups vs. placebo with respect to the serum vitamin E levels at 3 months (p-values 0.205, 0.0580, 0.0900, and 0.0572 for the 15mg/day, 30mg/day, 45mg/day and 60mg/day doses, respectively).

At nine months the comparisons between mean change in serum beta carotene levels in placebo to 15 mg/day, placebo to 45 mg/day, and placebo to 60 mg/day groups show a statistically significant decrease in mean serum vitamin E levels (p-values 0.042, 0.009, and 0.027 respectively). No significant differences between mean change in vitamin E levels between each of the supplementation groups is detected at three and nine months (p-values >0.05).

Comment [A16]: Are any of these differences that we would care about.

Based on the previous results, further analysis of vitamin E levels is done by presence of dosages of beta carotene supplementation grouped together for the same reasons as mentioned above.

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Exploratory analysis of area under the curve for beta carotene and vitamin E were conducted. Comparison between mean changes in serum beta carotene level and mean changes in serum vitamin E were done with respect to demographic variable categories. In addition, analyses were done to evaluate for effect modification or possible confounding on the relationship between beta-carotene dose and serum beta-carotene by sex, age divided by median of sample, BMI >25, cholesterol >200, and body fat percentage group divided by the median body fat percent.

Comment [A17]: This reads more like methods than results. You did not tell us what you found.

Discussion

In this randomized controlled trial, we evaluated the effect of oral beta-carotene supplementation on serum beta-carotene levels and levels of the associated lipid-soluble tocopherol, vitamin E. Our results support the conclusion that the administration of oral beta-carotene significantly increased serum beta-carotene at all doses of supplementation compared to placebo. There was a suggestion of dose-response, but significant differences of beta-carotene response at three and nine months were only seen between the highest and lowest doses of supplementation. Dose response at all levels was not found, although this may be due to insufficient power to detect effect. Although individual treatment doses had large confidence intervals of beta-carotene, this was improved when all treatment groups were combined, indicating that this was more an effect of the small number in subjects, rather than wide ranges of possible response.

Comment [A18]: You never even hinted at this in the Results.

Comment [A19]: Larger sample sizes will tend to decrease width of CI, while heterogeneity of mean response will tend to increase width of CI. It is hard to make much of this when using a t statistic.

Analysis of other characteristics of interest did not reveal any a statistically significant effect on response. There was suggestion of effect of sex on beta-carotene change at nine months in treated subjects. In the combined treated group, women compared to men had a greater increase in beta-carotene between months three and nine of borderline significance ($p = 0.0523$). BMI did not appear to influence results overall, but there was a trend towards increased serum beta-carotene in treated subjects with a normal BMI compared to those with high BMI (>25) ($p = 0.0655$). Percent body fat and cholesterol did not appear to have an effect on overall differences of beta-carotene at baseline and nine months. In that these were not part of our primary study aims, the data was not powered enough to fully address these questions.

Comment [A20]: If it is worthwhile to do an analysis, it is worthwhile to report the estimates. A report that is only the p values is nearly worthless. Review the lecture on the role of CI and p values.

There was a less clear effect of beta-carotene on vitamin E. An increase in vitamin E at three months was seen across all dose groups, including placebo. The subsequent decrease at nine months was more prominent and in the treatment groups, and was statistically significant compared to the placebo arm. Previous trials have not demonstrated a relationship between beta-carotene supplementation and change in vitamin E levels.[7] As this change occurred in placebo, it cannot be ascribed completely to beta-carotene supplementation. It is possible that a delayed effect of beta-carotene caused vitamin E levels to decrease in the treatment groups, but seasonal variation in diet or absorption caused an overall increase in all arms of randomization. Differences in diet among subjects also could also account for changes in vitamin E levels; this was also not recorded in our study. This may be a result of random error in measurements of subjects with no relationship between vitamin E and beta-carotene. Previous studies have documented diurnal as well as intra-individual variation in fat soluble vitamins.[8,9] These might have been less prominent in beta-carotene levels due to the magnitude of change from supplementation.

Comment [A21]: Interesting, but this only tells us the importance of having a placebo group.

Comment [A22]: And if this were the cause, you would need to know that all subjects started the study at similar times of year.

Comment [A23]: Do you mean differences in diet over time (as in the previous sentence) or do you mean that different subjects had different diets?

Comment [A24]: Yes, it could be "laboratory drift" over time, if all subjects started the study at approximately the same time.

It is possible that some form of effect modification may have been present in the relationship between beta-carotene and vitamin E. Given established relationships between lipid levels and vitamin E, we attempted to correct for variation in cholesterol. However, recalculation of vitamin E as a ratio to cholesterol also did not alter this observed relationship, nor did evaluation of correlation of differences of vitamin E and beta-carotene by cholesterol level. There is still a possibility that lipid changes had an effect of the observed outcome, in that cholesterol was not measured following randomization. Of interest, when the area under the curve of vitamin E was compared to that of beta-carotene in treated subjects, there was evidence of effect modification of BMI, with a positive relationship between beta-carotene and vitamin E for overweight and obese patients. This relationship was not observed in the differences of beta-carotene and vitamin E between 9 month and randomization, however. This may

Comment [A25]: Do you mean between beta-carotene levels and vit E or between beta-carotene supplementation and vit E levels?

Comment [A26]: Did you mention this analysis in the methods?

Comment [A27]: I think I am lost here.

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suggest that the AUC values better represented a difference in these levels over shorter time intervals than were seen in our sampling schedule of three and nine months post-randomization.

Further exploratory analysis into the response of vitamin E was not revealing. The small number of subjects in each treatment group limited such investigation into modifiers of dose response. Also, the small number of subjects in the placebo group limited in-depth evaluation of the fluctuating levels of vitamin E in absence of beta-carotene supplementation. Finally, limited time points of evaluation also did not allow us to fully examine the timing of vitamin E response to beta-carotene supplementation. As this study was unable to completely characterize these relationships, and thus further studies with specific outcome measures to identify these relationships would be appropriate.

In addition, specific disease outcomes may be of interest. Larger randomized trials, such as the proposed evaluation of beta-carotene on cancer incidence in the Physicians' Health Study will be useful in evaluating outcomes.[10] This will be important to further generalize the effect of supplementation in a wider population, given effects of smoking, alcohol, diet and co-morbidities on fat-soluble vitamins.

In this phase II trial of oral beta-carotene supplementation, we showed that oral supplementation is effective in increasing serum levels of beta-carotene. We were also observed an overall decrease of vitamin E levels seen in all arms, which was found to be more prominent in patients given beta-carotene supplementation. The causal pathway of this finding is unknown. It will be important to further examine this relationship to allow further study as to the outcomes of beta-carotene supplementation on cancer and heart disease.

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Table 1: Patient Characteristics at Randomization (by Dose Group)

		Placebo (Dose = 0)	Beta - Carotene 15 mg/day	Beta-Carotene 30 mg/day	Beta-Carotene 45 mg/day	Beta-Carotene 60 mg/day	Total
N		8	10	10	8	10	46
Sex	Male	5	5	3	4	5	47.8%
	Female	3	5	7	4	5	52.2%
Age (in years)	Mean (SD)	56.3 (4.3)	56.3 (4.6)	57.2 (4.1)	55.9 (3.1)	56.5 (5.2)	56.5 (4.2)
	Minimum	52	50	50	51	52	50
	Maximum	64	62	64	60	65	65
Weight (in lbs.)	Mean (SD)	180 (32.8)	167.8 (36.8)	151.8 (30.2)	172.6 (40.9)	159.4 (19.1)	165.5 (32.4)
	Minimum	118	118	123	126	126	118
	Maximum	220	213	204	253	190	253
BMI (in kg/m^2)	Mean (SD)	26.6 (3.6)	25.7 (3.6)	25.6 (2.6)	25.3 (3.3)	24.9 (2.4)	25.6 (3.03)
	Minimum	19.7	20.7	22.4	21.7	21.7	19.7
	Maximum	30.4	31.7	31.5	30.9	28.9	31.7
Cholesterol (in mg/dl)	Mean (SD)	217.8 (28.5)	223 (29.7)	213.2 (33.5)	213.3 (33.5)	238.1 (38.9)	221.5 (33.1)
	Minimum	190	171	159	169	209	159
	Maximum	283	265	268	263	312.5	312.5
Body Fat (in %)	Mean (SD)	28.0 (8.23)	27.6 (8.82)	30.3 (5.78)	32.5 (5.99)	30.4 (9.01)	29.8 (7.56)
	Minimum	17.0	15.8	21.5	26.9	17.8	15.8
	Maximum	41.6	44.5	37.0	42.5	42.5	44.5

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Table 2: Outcomes by Dose Group

		Placebo (Dose = 0)	Beta-Carotene 15 mg/day	Beta-Carotene 30 mg/day	Beta-Carotene 45 mg/day	Beta-Carotene 60 mg/day
N		8	10	10	8	10
Beta-Carotene (serum):						
Randomization	Mean (SD)	270.2 (136.3)	220.1 (127.9)	219.4 (83.8)	227.0 (105.5)	217.8 (122.3)
	Minimum	136.3	64.8	125.5	93.3	48.3
	Maximum	476.3	496	348.5	395.8	407.5
	Missing	0	0	0	0	0
After 3 months	Mean (SD)	243.5 (94.3)	1116.4 (317.4)	1302.3 (259.9)	1236.0 (239.3)	1466.7 (251.1)
	Minimum	109.3	699	854	860.5	1098
	Maximum	384	1602.7	1603.3	1440.5	1959.7
	Missing	0	0	0	0	1
After 9 months	Mean (SD)	186.3 (87.8)	1253.6 (570.5)	1504.6 (479.0)	1749.1 (579.0)	1877.6 (429.9)
	Minimum	84.5	576.8	849.3	950.3	1233.3
	Maximum	323	2018.8	2248.5	2310.4	2855
	Missing	1	2	1	1	1
Difference* (0 – 9 months)	Mean (SD)	-101.56 (66.8)	1048.2 (554.1)	1280.6 (429.3)	1522.3 (488.0)	1642.1 (392.8)
	Missing	1	2	1	1	1
Area Under The Curve	Mean (SD)	234.3 (91.3)	1131.8 (319.9)	1336.7 (271.9)	1324.3 (297.3)	1522.6 (249.5)
	Missing	0	0	0	0	1
Vitamin E (serum):						
Randomization	Mean (SD)	7.88 (1.42)	7.76 (1.21)	7.98 (1.62)	8.24 (0.95)	8.44 (1.27)
	Minimum	6.19	5.10	5.12	7.22	6.32
	Maximum	10.71	9.24	9.46	10.05	10.71
	Missing	0	0	0	0	0
After 3 months	Mean (SD)	8.27 (1.23)	8.71 (0.91)	9.15 (0.90)	8.98 (0.63)	9.11 (0.66)
	Minimum	6.50	6.36	7.12	7.89	8.07
	Maximum	10.11	9.74	10.55	9.78	10.02
	Missing	0	0	0	0	1
After 9 months	Mean (SD)	7.25 (1.13)	5.75 (0.50)	6.30 (1.14)	6.15 (0.88)	6.32 (1.12)
	Minimum	5.26	4.61	4.31	4.94	4.87
	Maximum	8.93	6.28	7.74	7.05	8.06
	Missing	1	2	1	1	1
Difference* (0 – 9 months)	Mean (SD)	-0.86 (0.63)	-1.74 (0.87)	-1.53 (1.60)	-2.18 (0.90)	-1.99 (1.14)
	Missing	1	2	1	1	1
Area Under The Curve	Mean (SD)	7.79 (1.12)	7.97 (0.86)	8.36 (1.12)	8.04 (0.49)	8.35 (0.74)
	Missing	0	0	0	0	1

* 9-month measurement minus baseline measurement

Comment [A28]: Any comments on continued increase in measurements over time? (Especially in light of your earlier reporting that effects of supplementation varies across subjects and your interest in effect modification by BMI, etc.

Comment [A29]: Any comments on extremely high values seen in some patients? Would there be concern about toxicity?

Comment [A30]: A good thing to include in this table along with the individual measurements. I note that min and max are just as important here, as toxicity can sometimes be better related to changes in some measurements, than to the absolute measurement itself. E.g., people can often do better with chronically low potassium than they can with sudden correction of those imbalances.

Comment [A31]: Given the missing value for individual measurements at 9 months, you might wonder how the AUC was calculated in that instance.

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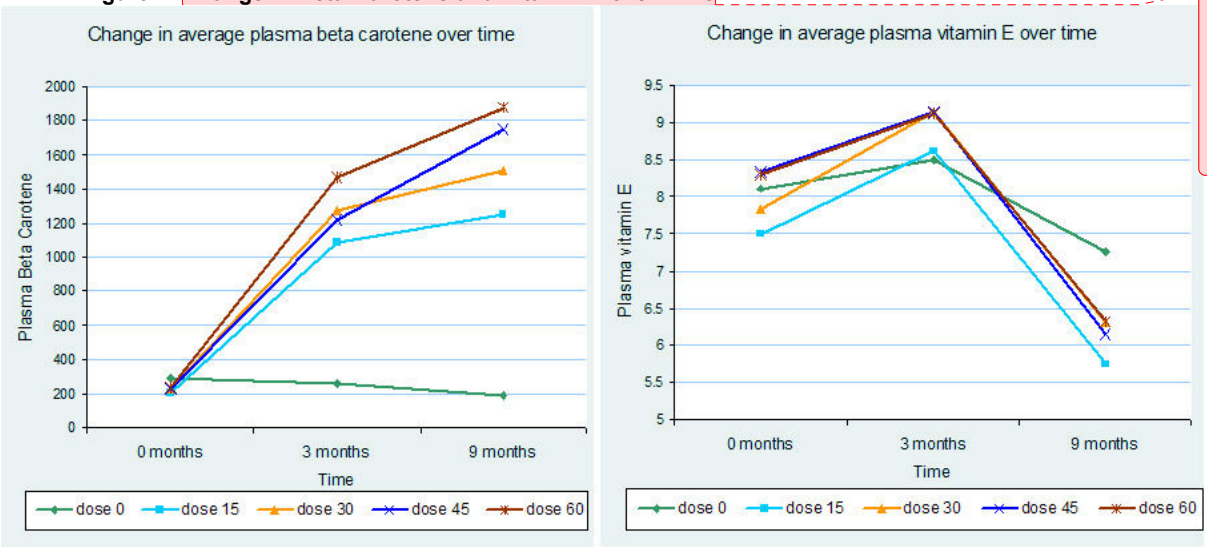
Table 3: Summary of Primary Results

	N	Mean	95% Confidence Interval	P (2-sided)
Change in Mean Plasma Beta-Carotene				
Baseline to 9 months*				
Placebo (dose = 0 mg/day)	7	-101.6	-163.3 to -39.8	
All other dose groups (15-60 mg/day)	33	1374.1	1196.7 to 1551.6	
Difference in Change of Means	40	-1475.7	-1659.6 to -1291.8	< 0.0001
Change in Mean Plasma Vitamin E				
Baseline to 9 months*				
Placebo (dose = 0 mg/day)	7	-0.86	-1.44 to -0.28	
All other dose groups (15-60 mg/day)	33	-1.85	-2.26 to -1.43	
Difference in Change of Means	40	0.98	0.32 to 1.64	0.0061
Baseline to 3 months+				
Placebo (dose = 0 mg/day)	7	-0.9	-1.4 to -0.3	
All other dose groups (15-60 mg/day)	33	-1.8	-2.3 to -1.4	
Difference in Change of Means	40	0.9	0.3 to 1.6	0.006

**9-month measurement minus baseline measurement*
+3-month measurement minus baseline measurement

Comment [A32]: These cannot be your primary results if you first compared all dose groups individually in order to decide whether to do this analysis.

Figure 1: Change in Beta-Carotene and Vitamin E over Time



Comment [A33]: Including SE bars would allow better use of these figures for inferential conclusions.

Alternatively, includind SD bars would allow better use of these figures for looking at potential toxicity

Either one would be acceptable.

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