Effect of Supplemental ß-Carotene on Serum Levels of ß-Carotene and Vitamin E: Phase II Clinical Trial

ABSTRACT

Background: ß-Carotene is a precursor of Vitamin A. Animal and observational studies have suggested it may be a chemopreventive agent. Studies have also indicated that supplemental ß-Carotene may affect serum vitamin E levels.

Objective: Assess the effect of supplemental ß-Carotene on serum ß-Carotene and serum Vitamin E levels.

Methods: A Phase II randomized double-blinded placebo controlled clinical trial included 46 adult volunteers randomly assigned to placebo or one of four doses of ß-Carotene (15, 30, 45, or 60 mg/day) for 9 months. Serum ß-Carotene, vitamin E levels and other characteristics were measured pre-randomization and at 3 and 9 months post-randomization. Baseline characteristics are described, and t-tests with unequal variances are used to compare 9-month mean serum ß-Carotene and vitamin E levels between subjects taking any dose of ß-Carotene versus placebo and for comparisons between adjacent dose groups. Comparisons at 3-months were also made.

Results: Mean serum ß-Carotene levels at 9 months increased significantly for all dose groups compared to the placebo group (mean difference of 1411 µg/L, 95% CI 1207.5-1615.5 µg/L, $p<0.0001$). Mean serum ß-Carotene for the 15 mg/day dose group was significantly higher compared to the placebo group (mean difference 1067 µg/L, 95% CI 588.1-1545.6 µg/L, $p<0.001$). No other statistically significant differences were detected for comparisons between adjacent dose groups. This increase in mean serum ß-Carotene was evident at 3 months when comparing any dose of ß-Carotene vs placebo. In terms of serum vitamin E levels, there was no significant difference at 3 months between any of the ß-Carotene supplementation groups, but at 9 months a significant difference in serum vitamin E levels was observed between all ß-Carotene supplementation groups compared to the placebo group (mean difference -1.12 mg/L, 95% CI -0.06 to -2.17 mg/L, $p<0.041$). There was also a statistically significant decrease in serum vitamin E between the placebo and 15 mg/day dose group (mean difference -1.50 mg/L, 95% CI -0.44 to -2.56 mg/L, $p=0.0115$), but no significant differences in serum vitamin E level were observed for any of the other comparisons between adjacent dose groups.

Conclusions: Supplementing with ß-Carotene increases serum ß-Carotene level and decreases serum vitamin E level over time. The lowest supplemental dose, 15 mg/day, was sufficient to significantly increase serum ß-Carotene after 9 months, though the concurrent decrease in vitamin E levels associated with supplementation requires further exploration.

Comment [A1]: prreventing what? (cancer was the interest of these researchers.

Comment [A2]: Actually it was plasma, but that was an error in my documentation

Comment [A3]: all dose groups combined? Make this clear

Comment [A4]: But were there differences between 15 and 60?

BACKGROUND

ß-Carotene is a free radical scavenger with anti-oxidative properties .[1, 2] It is one of 50 carotenoid pre-cursors to Vitamin A (retinol), forming 2 molecules of Vitamin A when cleaved by oxygen.[1] Approximately 15-30% of all carotenoids found in human serum are ß-Carotene, and it exists primarily as a lipid-soluble molecule in the blood.[2, 3] ß-Carotene, along with other carotenoids, are found naturally in colorful fruits, carrots, and green leafy vegetables.[3]

Interest in ß-Carotene as a chemopreventive agent has increased due to its anti-oxidative properties.[3, 4] Animal models have demonstrated that administration of ß-Carotene slows tumor growth.[5] Several studies have shown a potential correlation between low serum ß-Carotene levels and diagnosis with bladder cancer,[6] lung cancer,[7] breast cancer,[8] and some childhood cancers.[9] Based on these observations, it is hypothesized that dietary supplementation with ß-Carotene may prevent or slow certain types of cancer.[3, 4]

Prior studies have demonstrated significant increases in serum ß-Carotene levels with administration of purified ß-Carotene to human subjects.[10-15] In addition, one prior study found a significant correlation between baseline serum levels of ß-Carotene and vitamin E.[11] Studies in rats have shown that supplementing with large doses of vitamin E can decrease serum ß-Carotene levels and vice-versa.[16] Biologically, a similar effect in humans is plausible, and should be explored further. To date, an adequate dose of ß-Carotene necessary to significantly increase serum ß-Carotene levels has not been determined, and human studies have yet to determine if supplementation affects serum levels of other lipid soluble nutrients such as vitamin E.

METHODS

Questions of Interest. As the interest in supplemental ß-Carotene is due to its potential role as a cancer preventative agent, our primary goal is to determine the effect of supplemental ß-Carotene on serum ß-Carotene levels after the longest time period available for analysis. In addition, we aim to assess the effect of supplementation on serum vitamin E concentrations. Specifically, we focus on whether supplementation with ß-Carotene, at any dose and in a dose-response fashion, affects serum ß-Carotene levels and serum vitamin E levels. Secondarily, we evaluated the short-term effects of supplementation with ß-Carotene by determining the association between supplementation and serum ß-Carotene and supplementation and serum vitamin E levels at 3 months post-randomization.

We will address the following statistical questions:

Primary:

1.) Is there a difference in mean serum ß-Carotene levels for all dose groups combined compared to the placebo group at 9 months post-randomization?
2.) Is there a difference in mean serum ß-Carotene levels between adjacent dose groups at 9 months?

Secondary:

3.) Is there a difference in mean serum ß-Carotene levels at 3 months for all dose groups combined compared to the placebo group or when comparing adjacent dose groups?
4.) Is there a difference in mean serum vitamin E levels for all dose groups combined compared to the placebo group at 3 months and at 9 months post-randomization?
5.) Is there a difference in mean serum vitamin E levels between adjacent dose groups at 3 months and at 9 months?

We hypothesize that supplemental ß-Carotene will have a significant effect on serum ß-Carotene levels as early as 3 months and that the effect will continue to be evident at 9 months. In addition, we expect that higher doses of ß-Carotene will have a stronger effect on serum levels of ß-Carotene, but that this dose-response relationship will level-off after a certain dose. We also hypothesize that supplemental ß-Carotene will significantly affect serum vitamin E levels, but that this effect will also level-off after a certain dosage.

Data Source. This is a phase II, randomized, double-blinded, placebo controlled clinical trial. A total of 46 adult volunteers were randomly assigned to receive placebo or one of four doses of ß-Carotene (15, 30, 45, or 60 mg/day) for 9 months in a double-blinded fashion. At randomization, subjects' age, gender, weight, body-mass-index (BMI) (kg/m^2), body fat percent, serum levels of ß-Carotene (µg/L), vitamin E (mg/L) and cholesterol level (mg/dL) were determined. Serum levels of ß-Carotene and vitamin E were reassessed at 3 and 9 months post-randomization and an area under the curve (time average) calculated over the study period.

Statistical Methods. Based on the randomized nature of this study, we did not expect a difference in baseline measures between the treatment groups. To characterize the sample, we described the distribution of age, gender, weight, BMI, body fat percent, baseline levels of ß-Carotene, vitamin E and cholesterol for each group. The variability and skewedness of the distribution were evaluated for potential outliers and errors. Each variable was evaluated to identify any clinically relevant differences between groups. Stratified descriptive statistics are provided. For all analyses, point estimates, confidence intervals and two-tailed p-values are provided. 95% confidence intervals (95% CI) provide a range of values for the population parameter such that our observed data would be a typical sample of that population. P-values are utilized in hypothesis testing to determine the probability of observing such data given that the null hypothesis is true. We set significance at p-value <0.05 for all two-sided tests. Thus, we expect statistically significant results to occur less than 5% of the time when the null hypothesis is true.

The t-test with unequal variances was used to compare mean serum ß-Carotene levels between subjects taking any dose of ß-Carotene and subjects taking placebo at 3 months and at 9 months and to compare differences between adjacent dose groups. It was also used to compare mean vitamin E levels between subjects taking any dose of ß-Carotene and subjects taking placebo at 3 months and at 9 months and to compare differences

Comment [A5]: good to motivate

Comment [A6]: "following questions statistically:" Hopefully these are scientific questions.

Comment [A7]: This is probably of less interest for beta carotene, and instead the dose response is likely of greatest interest. It is not surprising that beta carotene supplementation would affect the plasma levels somehow.

Comment [A8]: Just looking at adjacent dose groups will not be very powerful. At least examine 60 vs 15 descriptively.

Comment [A9]: There is a better argument for this analysis with vitamin E.

Comment [A10]: and time?

Comment [A11]: skewness

Comment [A12]: This might be written to a client, but generally not to a journal audience, unless the data source were known to require extensive error checking and it were not your fault that there were errors

between adjacent dose groups. Due to the small sample size of this data set, the use of the t-test requires the assumption of normally distributed data. Measurements were analyzed without incorporating the baseline characteristics, presuming that randomization would minimize confounding between the various dosage groups. Data were analyzed using Stata versions 8.0 and 10.0 (StataCorp, College Station, Texas) and R version 2.6.0 (Free Software Foundation).

Comment [A13]: No, never. But always the t test assumes the approximate normal distribution of the sample means. And the heavier tails in the data the greater the sample size that would be required for this assumption to hold.

RESULTS

Baseline Demographics. Randomization of our 46 subjects resulted in 8 subjects in the placebo group and 38 subjects in the ß-Carotene group (10 subjects taking 15mg/day, 10 subjects taking 30mg/day, 8 subjects taking 45mg/day, and 10 subjects taking 60mg/day). One subject in the 15mg/day group was missing a baseline body fat percentage value; no other values were missing at baseline. For the outcome measures of interest, there were 6 subjects missing 9-month serum levels for ß-Carotene and vitamin E (two subjects in the 15mg/day group and one in each of the other dosage groups). We assumed that missing data represents non-informative censoring. Given the approximate uniform distribution across dosage groups we would not expect this assumption to affect our analysis.

Comment [A14]: This missing data pattern is NOT censoring. Censoring is a very special type of incomplete data.

Comment [A15]: Uniform distn of what? Missing cases?

The mean age in this sample of 22 males (48%) and 24 females (52%) was 56.5 years (SD 4.2 years). Baseline demographic data are provided in Table 1. There were no obvious outliers or issues of skewedness. The assumption of normally distributed data required for the t-test appears to be valid. Age and gender were similar across dose groups. Any differences in weight, BMI, body fat percentage and cholesterol were examined closely because the outcome variables are lipid soluble and may be affected by the volume of distribution of an individual's adipose tissue. The mean weight was slightly higher in the placebo and 45mg/day group but the range of BMI was similar across groups. Body fat percentage was much less variable in those subjects randomized to receive a 30mg/day dose of ß-Carotene. Baseline measures of cholesterol were variable in all groups, but the means were similar. Mean baseline ß-Carotene was similar across groups, except the mean was approximately 20% higher in the placebo group and mean baseline vitamin E level was slightly higher in the 45mg/day and 60mg/day groups. These variables did not appear skewed enough to warrant comparisons of geometric means or medians. Also, since the primary analysis compared 9-month levels without incorporating baseline levels, the mild differences between baseline ß-Carotene and vitamin E levels should not affect the analysis. Despite the small sample size, randomization appears to have equally balanced the groups. In addition, the data were analyzed per-protocol, though no efforts were made to assess compliance.

Comment [A16]: What would such issues have been? Probably I would just not have commented unless you did have some very outlying ages or something.

Comment [A17]: We want results here, not a travelogue. In methods you can explain why you did things, and in discussion you can talk about impact of findings.

Comment [A18]: Very small sample sizes makes it very hard to compare variances in a meaningful way.

Comment [A19]: Hopefully you made this decision before looking at the data. Had you noted problems with your chosen method of analysis, you might have added some exploratory analyses.

ß-Carotene Analysis. The mean difference in serum ß-Carotene level at 9 months between the placebo and all dose groups combined was 1411µg/L (95% CI 1207.5-1614.5 µg/L, p-value <0.0001) (Table 3). The comparisons of adjacent dose groups are shown in Table 3. There was a statistically significant difference between the mean serum ß-Carotene levels at 9 months for the placebo group compared to the 15 mg/day dose group (mean difference 1067 µg/L, 95% CI 588.1 to 1545.6 µg/L p-value <0.001). There were no statistically significant differences between the mean serum ß-Carotene level at 9 months between any of the other adjacent dose groups.

Comment [A20]: I don't understand this argument. If you had been incorporating baseline in some way, we woud then be less concerned about differences in baseline values.

Comment [A21]: You were acturally doing an intent to treat analysis. A per protocol analysis would take into account the compliance.

Comment [A22]: Mentioning the range of values would also be important to be able to assess possible toxicity,

At 3 months, a significant difference in serum ß-Carotene level was observed between the placebo and all dose groups combined (mean difference 1034 µg/L, 95% CI 916.2 to 1152.1 µg/L, p-value <0.0001) (Table 3). The 15 mg/day dose group demonstrated a mean difference in serum ß-Carotene level of 873 µg/L higher (95% CI 639.9 to 1105.8 µg/L, p-value <0.0001) compared to the placebo group. There were no statistically significant differences between the mean serum ß-Carotene level at 3 months between any of the other adjacent dose groups.

Vitamin E Analysis. Descriptively, mean and median serum vitamin E levels at 3 months increased in all groups, but at 9 months decreased in the placebo group and the treatment groups (Table 2). At 3 months there was no significant difference in vitamin E serum levels between the placebo and all dose groups combined (Table 3). At 9 months, the serum vitamin E level for those randomized to take ß-Carotene was significantly different compared to the placebo group. The mean vitamin E level was 7.3 mg/L in the placebo group and 6.1 mg/L in all dose groups combined, a difference of 1.2 mg/L (95% CI 0.062-2.170 mg/L, p-value=0.041).

Comment [A23]: Ranges would also be important here—did any particular individual have such low levels as to make us worry about toxicity? Perhaps the mean is lw because a couple individuals had severely low values.

Comparisons of the mean serum vitamin E concentration at 3 months and 9 months between adjacent dose groups are shown in Table 3. No significant differences at 3 months were found when comparing adjacent dose groups or when comparing the placebo group to all dose groups combined. As for 9-month comparisons, supplementation with 15 mg/day compared to the placebo group significantly affected serum vitamin E levels, resulting in a mean decrease of 1.5 µg/L (95% CI 0.44 to 2.56µg/L, p-value=0.0115). There were no significant differences in serum vitamin E levels between the other adjacent dosage groups.

Comment [A24]: As noted above, by only looking at adjacent dose groups, you are missing the fact that there is a trend in beta carotene across 15 – 60, but there was not much of a trend for vitamin E

DISCUSSION

This analysis has provided information about the association between ß-Carotene supplementation and serum ß-Carotene levels. At 9 months there was a significant difference in serum ß-Carotene level between the placebo group and those receiving any dose of ß-Carotene. Our analysis suggests that continuous ß-Carotene supplementation between 15mg/day and 60 mg/day tends to increase long-term ß-Carotene levels when compared to a placebo group. Thus, ß-Carotene supplementation is an effective means of increasing ß-Carotene concentration according to our analysis. Analyzing the difference in serum level between the placebo group and subjects who were randomized to receive a dose of 15 mg/day reaffirms this result, indicating that ß-Carotene supplementation tended to increase the level of serum ß-Carotene as early as 3 months, a trend which was also observed at 9 months compared to patients who received placebo. Further analysis demonstrated that there were no significant differences between any other adjacent dose groups at either of the time points investigated. Therefore, our analysis suggests that a supplemental ß-Carotene regimen of 15mg/day is sufficient enough to significantly increase the level of ß-Carotene in both the short and long-term.

Comment [A25]: Not only continued, but at 9 months levels were higher than at 3 mos among the supplemented groups. An important thing to note, because if we gave this for years, the levels might just keep increasing.

Secondarily, ß-Carotene supplementation has a delayed long-term effect on vitamin E levels as demonstrated by a significant difference in serum vitamin E levels between placebo and all dosage groups combined after 9 months, but not at 3 months. The analysis indicates that, in the long-term, mean vitamin E levels were significantly lower in those taking supplemental ß-Carotene compared to the placebo group. This suggests that taking a ß-Carotene supplement will tend to decrease the level of serum vitamin E, but that this effect may only be evident after an extended period of time. Analyses between adjacent dose groups showed that the 15 mg/day regimen significantly decreased vitamin E levels when compared to the placebo group after 9 months, but no significant differences were observed between subsequent dose groups. This indicates that after 9 months there is evidence of a decrease in serum vitamin E level from supplemental ß-Carotene at doses as low as 15mg/day, but that this decrease does not appear to be significantly amplified by increased supplemental dosages. Therefore, we expect that patients placed on ß-Carotene regimens will experience increased levels of serum ß-Carotene and decreased levels of serum vitamin E after 9 months.

Our results showing elevated serum ß-Carotene levels after supplementation in both the short term (3 months) and long term (9 months) are consistent with previous studies.[10-15, 17] Additionally, our results indicate that 15 mg/day supplemental ß-Carotene, the lowest dose administered, is sufficient to achieve significantly elevated serum levels of ß-Carotene. Studies have shown that the side effect of carotenodermia (development of orange skin shade) is unusual at this dose,[10, 12] enhancing its acceptability to patients. To our knowledge, no previous studies have explored the effects of ß-Carotene supplementation on serum vitamin E levels, though one showed a correlation in baseline levels between the two compounds and an inverse effect with vitamin E supplementation.[11] Our results indicate that the previously reported effects of ß-Carotene supplementation on serum vitamin E levels observed in rat models also exist in humans over a long-term period.[16]

Comment [A26]: Yes, but are they high enough for our purposes? Or perhaps too high?

The main limitation of this study is the small sample size. With only 8-10 participants per dose group, our power is limited to detect smaller differences in mean serum ß-Carotene between adjacent dose groups. Thus we first compared all ß-Carotene supplementation groups to the placebo group for both mean serum ß-Carotene and mean serum vitamin E levels to increase the power to detect a difference of supplementation. In order to effectively determine whether there is a difference between adjacent dose groups given larger doses, a larger sample size or more sophisticated statistical methods may be needed. In addition, we do not have information on patient compliance, though based on the elevated levels of ß-Carotene in groups assigned to supplementation, it is likely that compliance was not an issue. Furthermore, no information was provided about the seasons of the year during which the study was conducted or the amount of time over which study

Comment [A27]: I have not yet seen a clinical trial where compliance was perfect. But then again, noncompliance is real life.

Comment [A28]: Very good to note and discuss

participants were enrolled. In the placebo group, mean serum ß-Carotene level decreased over the course of the study. Serum ß-Carotene levels and serum vitamin E levels may fluctuate due to dietary variations based on seasonal availability of foods rich in these nutrients, which should be taken into consideration.

Based on the results of this phase II trial, a larger multi-center study should be designed with the knowledge that supplementing with 15 mg/day of ß-Carotene results in higher serum ß-Carotene levels after 9 months with a significant increase detected as early as 3 months. However, this dose also significantly decreased serum vitamin E concentrations after 9 months compared to placebo, and we would recommend further investigation with longer follow-up evaluation. Vitamin E levels may continue to decrease, and this possible toxic effect of supplementation should be assessed further. Seasonal variation and dietary intake of ß-Carotene should also be considered in future study designs. Finally, due to the interest in supplemental ß-Carotene as a potential cancer preventive agent, long-term studies designed to assess pertinent cancer outcomes should be planned.

References

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TABLES AND FIGURES

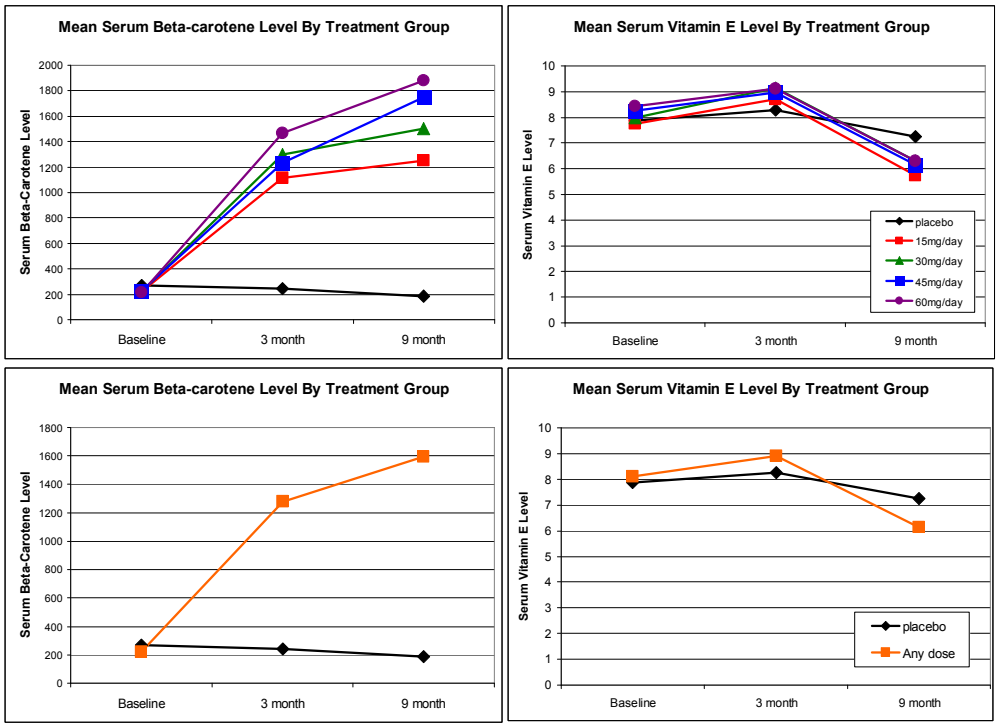
Table 1. Demographic descriptive statistics by treatment group (ß-Carotene dose mg/day)

	N	Mean	SD	Min	P25	median	P75	max
				Age (years)				
0	8	56.3	4.3	52.0	52.5	55.5	59.0	64.0
15	10	56.3	4.6	50.0	52.0	56.5	60.0	62.0
30	10	57.2	4.1	50.0	55.0	57.0	60.0	64.0
45	8	55.9	3.1	51.0	54.0	55.5	58.5	60.0
60	10	56.5	5.2	52.0	52.0	54.5	61.0	65.0
Total	**46**	**56.5**	**4.2**	**50.0**	**53.0**	**56.0**	**60.0**	**65.0**
				Weight (lbs)				
0	8	180.0	32.8	118.0	164.0	179.5	207.5	220.0
15	10	167.8	36.8	118.0	126.0	174.5	204.0	213.0
30	10	151.8	30.2	123.0	129.0	140.5	175.0	204.0
45	8	172.6	40.9	126.0	146.0	163.5	191.5	253.0
60	10	159.4	19.1	126.0	153.0	160.5	172.0	190.0
Total	**46**	**165.4**	**32.4**	**118.0**	**138.0**	**164.0**	**190.0**	**253.0**
				Body Mass Index (kg/m^2)				
0	8	26.55	3.64	19.68	24.30	27.22	29.63	30.45
15	10	25.69	3.58	20.69	22.34	26.57	27.73	31.68
30	10	25.57	2.65	22.36	24.03	25.07	26.28	31.55
45	8	25.35	3.32	21.66	22.41	25.07	27.66	30.86
60	10	24.94	2.43	21.67	23.05	24.77	25.67	28.95
Total	**46**	**25.59**	**3.03**	**19.68**	**23.15**	**25.41**	**27.68**	**31.68**
				Cholesterol (mg/dl)				
0	8	217.8	28.5	190.0	202.0	211.5	221.0	283.0
15	10	223.0	29.7	171.0	201.0	223.5	254.0	265.0
30	10	213.2	33.5	159.0	183.0	214.5	239.0	268.0
45	8	213.3	33.5	169.0	185.5	212.0	239.8	263.0
60	10	238.1	38.9	209.0	210.0	219.5	243.0	312.5
Total	**46**	**221.5**	**33.1**	**159.0**	**202.0**	**216.0**	**239.0**	**312.5**
				Body Fat (%)				
0	8	28.00	8.23	16.97	22.80	25.68	34.22	41.64
15	9*	27.56	8.82	15.76	21.52	27.24	31.79	44.51
30	10	30.34	5.78	21.52	25.36	30.83	36.21	37.01
45	8	32.46	5.99	26.9	27.62	30.07	37.47	42.50
60	10	30.39	9.01	17.78	19.61	32.79	37.16	42.50
Total	**45**	**29.76**	**7.56**	**15.76**	**24.85**	**30.62**	**36.14**	**44.51**
				Baseline Serum ß-Carotene Level				
0	8	270.24	136.29	136.25	148.08	227.75	398.88	476.25
15	10	220.06	127.94	64.75	136.00	185.63	237.75	496.00
30	10	219.35	83.85	125.50	140.00	205.00	282.25	348.50
45	8	226.98	105.54	93.25	147.92	216.38	299.13	395.75
60	10	217.81	122.34	48.25	98.25	224.29	310.75	407.50
Total	**46**	**229.35**	**112.54**	**48.25**	**140.00**	**212.00**	**310.75**	**496.00**
				Baseline Serum Vitamin E Level				
0	8	7.88	1.42	6.19	6.94	7.60	8.52	10.71
15	10	7.76	1.21	5.10	7.33	7.95	8.39	9.24
30	10	7.98	1.62	5.12	6.74	8.57	9.38	9.46
45	8	8.24	0.95	7.22	7.52	8.04	8.78	10.05
60	10	8.44	1.27	6.32	7.55	8.51	9.31	10.71
Total	**46**	**8.06**	**1.29**	**5.10**	**7.22**	**8.11**	**9.04**	**10.71**

	Gender	
	Male N (%)	**Female N (%)**
0	5 (63%)	3 (37%)
15	5 (50%)	5 (50%)
30	3 (30%)	7 (70%)
45	4 (50%)	4 (50%)
60	5 (50%)	5 (50%)
Total	**22 (48%)**	**24 (52%)**

* One patient in the 15mg/day group was missing information on baseline percent body fat.

Figure 1. Comparison of serum ß-Carotene and vitamin E levels by dosage group over the entire study period.



Comment [A29]: I would give some indication of the range of measurements in the top panels, and I would let the top panels speak for the combined group analysis, and just omit the lower panels.

Note the dose response among the higher doses and the continued increase over time. You commented on neither of these. You should have.

Table 2: Descriptive statistics for the serum ß-Carotene and serum vitamin E levels at 3 months and at 9 months post-randomization by treatment group (ß-Carotene dose mg/day)

	N	Missing	Mean	SD	Min	25th %	50th %	75th %	Max
Serum ß-Carotene Levels – 3 Months									
0	8	0	243.5	94.3	109.3	179.7	220.5	327.3	384.0
15	10	0	1116.4	317.4	699.0	745.0	1203.0	1334.3	1602.7
30	10	0	1302.3	259.9	845.0	1172.0	1289.3	1540.5	1603.3
45	8	0	1236.0	239.3	860.5	1034.8	1343.3	1415.7	1440.5
60	9	1	1466.7	251.1	1098.0	1292.0	1410.3	1595.3	1959.7
Serum ß-Carotene Levels – 9 Months									
0	7	1	186.3	87.8	84.5	126.0	149.0	286.0	323.0
15	8	2	1253.6	570.5	576.8	695.4	1250.0	1771.2	2018.8
30	9	1	1504.6	479.0	849.3	1157.3	1498.5	1840.0	2248.5
45	7	1	1749.1	579.1	950.3	993.0	1848.3	2247.7	2310.4
60	9	1	1877.6	429.9	1233.3	1724.7	1865.0	1917.7	2855.0
Serum Vitamin E Levels – 3 Months									
0	8	0	8.273	1.232	6.497	7.337	8.400	9.052	10.113
15	10	0	8.709	0.908	6.360	8.630	8.848	8.987	9.737
30	10	0	9.152	0.899	7.123	8.810	9.415	9.553	10.547
45	8	0	8.977	0.633	7.885	8.688	8.805	9.583	9.780
60	9	1	9.114	0.662	8.067	8.567	9.263	9.613	10.017
Serum Vitamin E Levels – 9 Months									
0	7	1	7.253	1.125	5.258	6.951	7.230	7.725	8.933
15	8	2	5.753	0.501	4.61	5.718	5.836	6.009	6.282
30	9	1	6.295	1.136	4.313	5.610	6.197	7.487	7.735
45	7	1	6.147	0.876	4.935	5.573	5.948	6.973	7.053
60	9	1	6.315	1.115	4.867	5.523	5.933	7.200	8.057

Comment [A30]: Would this be a dangerously high level? You probably don't know, but it is still worth commenting on.

Comment [A31]: Would this be a dangerously low level?

Table 3: Results of comparative analyses between dose groups at 3 months and at 9 months including point estimates of the difference in means between groups, 95% CI, and two-sided P-values, by serum ß-Carotene and Vitamin E levels

Comparison Group	Point estimates	95% CI	P-value
Serum ß-Carotene Levels at 3 months			
All dose groups vs. placebo	1034	916.2, 1152.1	<0.0001
15mg/day vs. placebo	873	639.9, 1105.8	<0.0001
30mg/day vs. 15mg/day	186	-87.3, 459.2	0.1695
45mg/day vs. 30 mg/day	-66	-316.8, 184.3	0.5822
60mg/day vs. 45 mg//day	231	-23.2, 484.5	0.0719
Serum ß-Carotene Levels at 9 months			
All dose groups vs. placebo	1411	1207.5, 1615.5	<0.0001
15mg/day vs. placebo	1067	588.1, 1545.6	<0.001
30mg/day vs. 15mg/day	251	-301.5, 803.6	0.346
45mg/day vs. 30 mg/day	244	-348.0, 836.9	0.385
60mg/day vs. 45 mg//day	129	-448.8, 705.9	0.633
Serum Vitamin E Levels at 3 months			
All dose groups vs. placebo	-0.71	-1.75, 0.33	0.154
15mg/day vs. placebo	-0.44	-1.57, 0.70	0.419
30mg/day vs. 15mg/day	-0.44	-1.29, 0.41	0.288
45mg/day vs. 30 mg/day	0.17	-0.59, 0.94	0.636
60mg/day vs. 45 mg//day	-0.14	-0.81, 0.53	0.669
Serum Vitamin E Levels at 9 months			
All dose groups vs. placebo	-1.12	-0.06, -2.17	0.041
15mg/day vs. placebo	- 1.50	- 0.44, - 2.56	0.0115
30mg/day vs. 15mg/day	0.54	-0.37, 1.46	0.2202
45mg/day vs. 30 mg/day	-0.15	-1.23, 0.93	0.7725
60mg/day vs. 45 mg//day	0.17	-0.90, 1.24	0.7403