β -carotene and Vitamin E, A Phase II Trial – Group 13

Summary

In this phase II randomized clinical trial, volunteer subjects were assigned in a double blinded manner to a treatment or placebo to assess whether there were toxicities associated with dietary intake of β-carotene. This trial consisted of 46 individuals assigned to one of five treatments of orally administered β-carotene, with doses of 0, 15, 30, 45 and 60 mg/day for 9 months. The investigators are interested in the serum levels of vitamin E and β-carotene after 3 and 9 months post randomization, and specifically if there is any change in vitamin E.

Blood serum levels of both vitamin E and β-carotene were analyzed along with measurements of other parameters taken at baseline, such as sex, weight, body mass index, percent fat and serum cholesterol. In the 0 to 9 month analysis, all doses of β-carotene led to a decrease in vitamin E, however the only non-statistically significant group was for the 30 mg/day treatment group. In the 0 to 3 month analysis, there were no significant associations.

The 95% confidence interval for change in β-carotene with doses of 15, 30, 45 and 60 mg/day where $p<0.05$, in the 0-3 month analysis are, (697 - 1149 mcg/L), (949 -1270 mcg/L), (687 – 1183 mcg/L) and (1065 -1450 mcg/L) respectively. In the 0-9 month analysis, for all doses $p<0.05$, and the 95% confidence interval for doses of 15, 30, 45 and 60 mg/day β-carotene are (686 -1614 mcg/L), (1050 – 1714 mcg/L) and (1172 – 2075 mcg/L) respectively.

The 95% confidence interval (p-value not significant) for change in vitamin E with doses of 15, 30, 45 and 60 mg/day in the 0-3 month analysis are (-0.26 - 1.36 mg/L), (-0.05 - 1.59 mg/L), (-0.4 – 107 mg/L) and (-0.53 - 1.35 mg/L) respectively. In the 0-9 month analysis for change in vitamin E, the 95% confidence interval and p-value are (-1.72 – (-0.36) mg/L, $p<0.05$ for 15mg/dl), (-1.96 - 0.61 mg/L, p not significant for 30mg/dl), (-2.24 – (-0.4) mg/L, $p<0.05$ for 45 mg/dl) and (-2.1 – (-0.15) mg/L, $p<0.05$ for 60 mg/dl).

Comment [A1]: what treatment

Comment [A2]: no, it was supplementation. very different from diet, because other nutrients are correlated with dietary intake.

Comment [A3]: it was actually plasma, but that was erroneous in my documentation

Comment [A4]: not interested more than beta carotene, but in addition

Comment [A5]: We cannot interpret these without knowing what the placebo group did. Comparison across dose groups is the major thing we are interested in. We randomized dose, we did not randomize time.

Background

β-carotene is found in green and yellow vegetables and is a precursor of vitamin A, which has multiple beneficial roles in human health.[1] β-carotene itself is also a potent antioxidant.[1,2] Because of its antioxidant properties, β-carotene has been investigated as a cancer prevention agent.[4-6] The best epidemiologic evidence for positive health effects of β-carotene is for a diminished risk of lung cancer for patients with a higher dietary intake of β-carotene.[5] β-carotene levels in blood and adipose tissue reflect the amount of β-carotene that has been ingested over the preceding few weeks.[1]

Comment [A6]: And (unrelated to this project) this is why it is so interesting that the RCTs done later show harmful effects of beta carotene.

Vitamin E is another lipid-soluble vitamin that helps maintain the fluidity of cellular membranes. It is found in many foods, including soybeans, many oils, meats, nuts, and cereal grains.[6] Its main role also is as an antioxidant, and it may provide some protection from cancers and/or heart disease.[5,10] There have been a few reports of the bioavailability and storage of fat-soluble vitamins (such as vitamin A and E) being affected by other fat-soluble vitamins or carotenoids, such as β-carotene.[7] One theory is that fat-soluble vitamins may decrease the levels of other fat-soluble vitamins via competition for absorption sites in the intestine or storage sites in the liver.[7] At least one study in rats indicated that supplementation with β-carotene is associated with lower vitamin A and E levels.[8]

If administration of β-carotene were to diminish the serum level of vitamin E, any possible benefit from β-carotene supplementation may come at the cost of losing benefits of vitamin E. Investigating the effect of β-carotene on vitamin E is imperative prior to recommending β-carotene supplementation to the general public.

Questions of Interest

The questions of interest for this project were: (1) to measure the response of plasma levels of β-carotene to different β-carotene doses after 3 months and after 9-months of treatment, and (2) to measure the effect of β-carotene administration on vitamin E levels after 3 months and after 9 months of treatment with β-carotene. We hypothesized that β-carotene supplementation will lower plasma vitamin E levels.

Comment [A7]: Perhaps you did, but I think that was a rather strong statement. I would say that I wanted to find out whether it did. I do not always have to guess which way it would go for a safety endpoint.

Source of the Data

To determine reliable values representative of the general population for analyses of β-carotene and Vitamin E, a review of the variation from biological and pathological influences was conducted to frame the scientific approach to measuring blood serum parameters for β-carotene and α-tocopherol. The choice of α-tocopherol as a biomarker to measure vitamin E arises from its ubiquity and concentration in blood over other tocopherols and tocotrienols (the two chemical classes of the constituents we call vitamin E).

Blood samples were collected for assay and relevant biological and clinical data such as age, sex, weight, body mass index (BMI), percent body fat at randomization and cholesterol were collected. BMI relates a person's weight to their height and is reported in kg/m^2, and cholesterol is measured in blood serum (mg/dl). These variables were considered because of suggestions about their role as effect modifiers or confounders in β-carotene and α-tocopherol levels in serum. Missing data points prevented the inclusion of several observations in our analysis, however there was no impact on the baseline data. The missing data was not patterned or consistent with sampling bias or drop-out due to excessive toxicities.

Comment [A8]: There is nothing in your data to tell you whether the dropout was due to toxicities. So you need to be a little more circumspect in your wording here. Perhaps, there was nothing to suggest that dropout was more frequent at higher doses, as might be expected if it were due to toxiciitiies.

The study consisted of 46 volunteers randomly assigned to receive one of five treatment doses for nine months in a double blind clinical trial. Samples of venous blood were taken from the fasting subjects to be analyzed for the purpose of this study. Samples were collected in tubes containing an anticoagulant (lithium heparinate) and analyzed by High Performance Liquid Chromatography (HPLC) for β-carotene and α-tocopherol. Because of the randomly assigned treatments there is little expectation of confounding by unforeseen variables.

Comment [A9]: All of this is news to me, but I don't usually pay attention to such things. Parenthetically, I will note the habit of many basic scientists to give me information about the brand name of the syringe used to inject mice, and then never tell me how many mice were used. If these are standard methods, why talk about them? If they were nonstandard, then discuss their possible impact.

Methods

Two levels of analysis were performed with this data set. First, common techniques of exploratory data analysis, including the inspection of descriptive statistics and scatterplots, were applied to justify any assumptions made in the subsequent analysis. Next, two-sample t-tests assuming unequal variance served as inferential analysis between dose groups in order to address the two scientific questions discussed above (after taking the change of level of each variable, we should not assume equal variance when performing the statistical tests).

Comment [A10]: I agree we should not assume equal variances, but it is not clear that that decision is at all different because we were taking the change as our primary outcome.

Issues which have been considered in the exploratory analysis include handling of missing data, multiple comparisons, transformation, and presence/absence of confounders/precision variables/effect modifiers. Comparison of baseline variables including age, gender, weight, body mass index, cholesterol, percentage fat, vitamin E level, and β-carotene levels, were used to assess whether unforeseen differences exist between dosage groups at the start of randomization.

As association between variables might still exist in this randomized study of 46 participants, scatter-plots and descriptive statistics were used as diagnostic tools to assess such level. The main interest was to ensure that dosage levels were not associated with variables like

Comment [A11]: I would argue that randomization was "to ensure" and your data analysis here was "to assess whether"

gender, body mass index, and cholesterol, which were previously reported to be possibly casually associated with vitamin E and β-carotene, thus were used to evaluate the existence of confounders and effect modifiers. Precision variables were considered, but not used in analysis. Small sample size prevented meaningful subgroup analyses. In order to gain insight in whether missing data could be informative, distribution of missing data were inspected by basic descriptive statistics in order to determine whether they preferentially lay in some dosage subgroups that could be clinically relevant.

Determination of dosage effect of vitamin E and β-carotene were tested by comparing difference of change of each variable between months of interest. Comparing the difference of change allows adjustment for levels of β-carotene and vitamin E that may vary independent of supplementation because of seasonal variation of intake in food rich in β-carotene and vitamin E, respectively.[6] The primary interest was to estimate the true difference of change of serum β-carotene and vitamin E of different dose groups between 3 months or 9 months and baseline.

Analysis was performed in Stata10 (College Station, Texas).

Comment [A12]: This is jargon that would not be understood by the casual reader. Instead you would have to say something like "We considered whether adjusting for certain variables would increase precision of our analysis." The common person does not know what a "precision variable" is.

Comment [A13]: This wording is unclear. I presume you meant that you compared dose groups with respect to the change in plasma levels.

Results

Table 1 shows the baseline descriptive statistics for the study population. As expected, distribution of age, gender, weight, body mass index, cholesterol level, and percentage body fat across dosage group at randomization, as detected by inspecting their respective means, median, standard deviation, and interquantile range (when applicable), do not differ largely between groups even in this sample of 46 volunteers.

Patient ages ranged from 50 to 65 years old, with approximately 63% men in placebo group, 50% in 15, 45 and 60 dose groups, and 30% for 30 dose group. There were 8 patients assigned to the placebo group, 10 to the 15 mg/day, 10 to the 30 mg/day, 8 to the 45 mg/day, and 10 to the 60 mg/day beta-carotene dose group.

At time of randomization, the average β-carotene measurement in the placebo group was 270.24 mcg/L (SD 136.29 mcg/L), in 15 mg/day dose group – 220.06 mcg/L (SD 127.94 mcg/L), in 30 mg/day dose group – 219.35 mcg/L (SD 83.85 mcg/L), in 45 mg/day dose group – 226.98 mcg/L (SD 105.54 mcg/L), in 60 mg/day dose group – 217.81 mcg/L (SD 122.34 mcg/L). The average vitamin E measurement at time of randomization in the placebo group was 7.88 mg/L (SD 1.42 mg/L), in 15 mg/day dose group – 7.76 mg/L (SD 1.21 mg/L), in 30 mg/day dose group – 7.98 mg/L (SD 1.62 mg/L), in 45 mg/day dose group – 8.24 mg/L (SD 0.95 mg/L), in 60 mg/day dose group – 8.44 mg/L (SD 1.27 mg/L).

For each baseline variable, the mean and median between dose groups at baseline seem to be quite similar (which means that the variables are not skewed). It should also be noted that variability seems to be quite large among population in serum β-carotene level, in which standard deviation is nearly one hundred. Also it should be noted that there does not seem to be outliers in both serum β-carotene and vitamin E level. But there seems to be an outlier in the weight variable (253 lbs).

With increased time on study, the number of missing observations increases: for β-carotene placebo, 30, 45 and 60 mg/day dose groups have one missing value each; there are two missing values for the 15 mg/day dose group. For vitamin E there is one missing point for each of placebo, 45 and 60 mg/day dose groups, and two missing points for 15 and 30 mg/dose groups. Patients who dropped out of the study were not different in terms of age, sex and baseline β-carotene and vitamin E levels compared to those patients who remained in the study.

Comment [A14]: For what it is worth, the means are probably the best indicator of whether there would be confounding. Ranges might tell us about influential subjects. More next quarter on these two topics.

Comment [A15]: I would have given the sample sizes in each treatment group before I subclassified them by sex within each dose group.

Comment [A16]: Probably sufficient to let a table give these particulars, though in the text you might want to talk about the overall mean and/or the range of means across groups.

Comment [A17]: Unclear the relevance to our scientific question

Comment [A18]: personally I don't have a way to judge whether this is large or not. I do note that people tend to binge on beta-carotene, so there is a lot of within person variability over time, as well as a lot of between person variability.

Comment [A19]: do

Comment [A20]: Good to note. Doesn't prove anything, but we do sleep easier knowing that we could not detect trends in the probability of dropout.

Statistics for β-carotene and vitamin E at time of randomization (baseline), at month 3 and month 9 of treatment as well as area under the curves are presented in Table 2. We can see similarity of β-carotene and vitamin E levels for dose groups for different time points. There is a general trend towards increasing mean of β-carotene level in all dose groups with time on supplement. At 9 months, the change in vitamin E tends to be lower in the treatment group than the placebo group. Such decreasing trend does not seem to be observed 3 months into the study. It should be noted that the decreasing relationship in 9 months does not look that noticeable. These trends can also be seen in Figure 1 (each point in the graph represents the difference of 9 month or 3 month and the baseline level). Comparison of the area under the curve for β-carotene and vitamin E levels agrees with our result. The results of the analysis of dosage effect on plasma β-carotene and vitamin E are also presented in table 3 and include point estimates, standard error, a 95% confidence interval and the p-value for inferential statistics for the change in vitamin E and β-carotene by time of analysis.

Administration of β-carotene at 60 mg/day dose group shows increase in serum β-carotene from beginning to 9th month measurements of 1743.71 mcg/L (95% CI: 1439.83 to 2047.58; p<0.0001). Similar effect of increasing in serum β-carotene was observed also for all other dose groups (see Table 3). At 3rd month of treatment, adjusted to baseline there was less significant increase in serum β-carotene for the 60 mg/day group – 1257.9 mcg/L (95% CI: 1065.39 to 1450.41; p<0.0001), same effect was observed for all dose groups. The 60 mg/day supplementation results in the biggest increase of plasma β-carotene.

The effect of β-carotene supplementation at 15 mg/day, 45 mg/day and 60 mg/day shows decreasing serum vitamin E level from beginning to 9th month measurements by 0.88 mg/L (95% CI: decreasing by 0.36 to 1.72; p=0.0420), 1.32 mg/L (95% CI: decreasing by 0.4 to 2.24; p=0.0092) and 1.12 (95% CI: -0.15 to -2.1; p=0.0266) respectively. However, there was no difference in mean serum vitamin E level adjusted to baseline at 9 months for 30 mg/day dose groups (-0.67, 95% CI: -1.96 to 0.61; p= 0.2743). Similarly, after 3 months of treatment, there was no significant change in serum vitamin E for any dose of β-carotene (see Table 3).

Comment [A21]: You would need to define this term for your intended audience. (Pharmacokineticists would be used to this term, but they might expect it to be a measurement within each individual over a shorter period of time.)

Comment [A22]: Even placebo?

Comment [A23]: what is "the treatment group". I had 5 of them in my dataset—4 active supplementation and 1 placebo. Your methods did not make clear that you might be combining them.

Comment [A24]: In fact, at 3 months all dose groups tended to have higher values.

Comment [A25]: Do you think you gained or lost precision by analyzing the change rather than the absolute measurements? You certainly lost the ability to assess individual toxicity that might be related to absolute levels (though there are times that toxicity is related more to the change than it is to the absolute level.)

Comment [A26]: We would want descriptive statistics, too. Not just inferential statistics.

Discussion

In this analysis, we characterized the response of plasma β -carotene levels after 3 and 9 months of administration of β-carotene to adults. Plasma β-carotene level increased by a factor of 5-8 after 3 and 9 months, regardless of dose group. We also showed that vitamin E levels decreased approximately 15% in response to the administration of β-carotene at 9 months. This association was not found after 3 months of therapy. These results are seen in Figure 1, in which the red (lower) line illustrates a negative change from baseline for each dose group other than placebo. The blue (upper) line is the difference at 3 months for each dose group, and is not different from the baseline value.

This decrease in vitamin E has important implications as studies move forward in defining a possible role of β-carotene supplementation on cancer prevention. Possible mechanisms include competition between β-carotene and vitamin E for absorption sites in the intestine or for storage sites in fat and the liver.[7] Similar results have been described for rats: after rats were administered a diet of β-carotene for 17 weeks, their plasma vitamin E levels were halved. The authors hypothesized that β-carotenes competes with fat-soluble vitamins for absorption in the intestine, transport in blood, and storage in tissues.[8]

Change in plasma β-carotene level was of a similar magnitude after 3 and 9 months,

Comment [A27]: You suddenly switch from differences to ratios. Is there a reason? Do you have inference to go with the ratios? That is, do you have CI for the ratios.

And it was not regardless of dose group and not regardless of time. That is, there was a major difference between placebo and dose 15, but there were also trends by dose from 15 – 60. And it looks to me like there is a difference between 3 mos and 9 mos in the magnitude of the difference.

And how did you compute your ratios? Are they differences between 9 mos and baseline within each dose group (bad) or comparing each dose group to placebo (good). We randomized dose, not time. In attributing differences to treatment, we **MUST** consider comparisons to what would have happened otherwise. The placebo group tells us that.

regardless of dose group. Further studies should be done to determine if side effects differ between dose groups. Skin discoloration has been noted after several weeks of administration of 24-36 mg of β-carotene, which could potentially be a cause for patients to discontinue the drug. Subject drop-outs were similar at the 9 month mark across dose groups, so side effects might have been minimal, though we have not collected any data which probed for any side effects other than drop in vitamin E level.

The association between β-carotene administration and decreased plasma vitamin E levels at 9 months was observed for groups administered 15, 45, and 60 mg/day of β-carotene, with a similar magnitude of change between groups. However, the association was not observed for the group administered 30 mg. The change in vitamin E overall is very small, so we may have simply had a type II error. The small numbers in each group magnifies this risk. Additionally, among all of the patients in our study, only two had a vitamin E level increase over the study period, and the patient with the largest increase (1 mg/L) is in the 30 mg group. This may have been a true increase for reasons not accounted for in our data (e.g., seasonal variation in foods that are rich in vitamin E, such as apples, tomatoes, or sweet potatoes), or an error in measurement. Despite this discrepancy, it is otherwise clear that an inverse association between plasma β-carotene level and plasma vitamin E level exists.

Our study has several limitations. There are few participants, increasing our chance of missing a true association. We are also unable to perform meaningful subgroup analysis because of the small number of patients. Without data at additional time points, we cannot say what the duration of β-carotene administration is that is necessary to change vitamin E levels. Further study is needed to determine the exact relationship between levels of β-carotene and vitamin E.

β-carotene levels increase by a similar magnitude after 3 and 9 months of administration of β-carotene of doses 15-60 mg/day. We have also shown that administration of β-carotene is associated with a decrease in vitamin E levels at 9 months. It is possible that diminished vitamin E levels may have adverse effects and should be further investigated prior to recommending β-carotene supplementation to the public as a means to prevent cancer. Further studies should be undertaken to further delineate this relationship.

Comment [A28]: I disagree. Where did you look at this specifically

Comment [A29]: You do not have this data from this study, so you should make clear that "In other studies…"

Comment [A30]: Do not confuse lack of statistical significance with proof of no association. Your next sentence is all important. I would not have singled out the 30 mg group in this way.

Comment [A31]: (There are some who would wish that subgroup analyses could never be performed, because they are always overinterpreted. But I do agree that we should try to look at them.)

Comment [A32]: For what it is worth, I really have monthly measurements. And I personally am worried that the continued increase from 3 mos to 9 mos means that there might be significant toxicities had we gone for a longer period of time.

Comment [A33]: Yes. And it is this that makes all the more egregious your omission of providing us information on an individual level about how low vitamin E might have gone in a few individuals.

References

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Table 1: Summary of patient characteristics by dose of β-carotene (treatment groups) at baseline.

Dose group (mg/d)	Baseline variable				
	Age (years)				
	N	Mean (SD)	Minimum	Median	Maximum
0	8	56.3 (4.3)	52	55.5	64
15	10	56.3 (4.6)	50	56.5	62
30	10	57.2 (4.1)	50	57	64
45	10	55.9 (3.1)	51	55.5	60
60	10	56.5 (5.2)	54	54.5	65
	Weight (lbs)				
	N	Mean (SD)	Minimum	Median	Maximum
0	8	180.0 (32.8)	118	179.5	220
15	10	167.8 (36.8)	118	174.5	213
30	10	151.8 (30.2)	123	140.5	204
45	10	172.6 (40.9)	126	163.5	253
60	10	159.4 (19.1)	126	160.5	190
	BMI (kg/m^2)				
	N	Mean (SD)	Minimum	Median	Maximum
0	8	26.5 (3.6)	19.7	27.2	30.4
15	10	25.7 (3.6)	20.7	26.6	31.7
30	10	25.6 (2.6)	22.4	25.1	31.6
45	10	25.3 (3.3)	21.7	25.1	30.9
60	10	24.9 (2.4)	21.7	24.8	28.9
	Cholesterol (mg/dl)				
	N	Mean (SD)	Minimum	Median	Maximum
0	8	217 (28)	190	211.5	283
15	10	223 (30)	171	223.5	265
30	10	213 (33)	159	214.5	268
45	10	213 (34)	169	212	263
60	10	238 (39)	209	219.5	312
	Percent Body Fat				
	N	Mean (SD)	Minimum	Median	Maximum
0	8	28 (8)	17	26	42
15	10	28 (9)	16	27	45
30	10	30 (6)	22	31	37
45	10	32 (6)	27	30	43
60	10	30 (9)	18	33	43

Table 2, Descriptive statistics for outcomes by treatment group

	Dose group	N	Mean (mcg/L)	SE	95% Confidence Interval			Dose group	N	Mean (mg/L)	SE	95% Confidence Interval	
β -carotene							Vitamin E						
Baseline	**0**	8	270.24	48.19	156.3	384.18	**Baseline**	**0**	8	7.88	0.5	6.69	9.06
	15	10	220.06	40.46	128.53	311.59		**15**	10	7.76	0.38	6.89	8.62
	30	10	219.35	26.52	159.37	279.33		**30**	10	7.98	0.51	6.83	9.14
	45	8	226.98	37.31	138.75	315.21		**45**	8	8.24	0.33	7.45	9.04
	60	10	217.81	38.69	130.29	305.33		**60**	10	8.44	0.4	7.53	9.35
3 month	**0**	8	243.52	33.35	164.65	322.39	**3 month**	**0**	8	8.27	0.44	7.24	9.3
	15	10	1116.37	100.36	889.34	1343.39		**15**	10	8.71	0.29	8.06	9.36
	30	10	1302.32	82.18	1116.4	1488.23		**30**	10	9.15	0.28	8.51	9.8
	45	8	1236.04	84.62	1035.95	1436.13		**45**	8	8.98	0.22	8.45	9.51
	60	9	1466.67	83.71	1273.62	1659.71		**60**	9	9.11	0.22	8.61	9.62
9 month	**0**	7	186.32	33.18	105.12	267.52	**9 month**	**0**	7	7.25	0.43	6.21	8.29
	15	8	1253.58	201.69	776.66	1730.51		**15**	8	5.75	0.18	5.33	6.17
	30	9	1504.61	159.68	1136.4	1872.82		**30**	8	6.3	0.38	5.42	7.17
	45	7	1749.08	218.86	1213.55	2284.61		**45**	7	6.15	0.33	5.34	6.96
	60	9	1877.63	143.29	1547.2	2208.07		**60**	9	6.32	0.37	5.46	7.17
Area under the curve	**0**	8	234.34	32.29	157.98	310.7	**Area under the curve**	**0**	8	7.79	0.4	6.86	8.73
	15	10	1131.81	101.15	902.98	1360.63		**15**	10	7.97	0.27	7.36	8.58
	30	10	1336.67	85.98	1142.17	1531.17		**30**	10	8.36	0.36	7.55	9.16
	45	8	1324.3	105.1	1075.78	1572.81		**45**	8	8.04	0.17	7.63	8.44
	60	9	1522.56	83.18	1330.76	1714.36		**60**	9	8.35	0.25	7.79	8.92

Comment [A34]: It is **VERY IMPORTANT** to give min and max here. We might worry about individual toxicities. You gave us no indication of that in this table (though the changes are in your graph—but even then, we can't tell if there were levels that were too high or too low on an absolute scale).

Comment [A35]: Note this decrease over the course of the study. Shows the importance of a placebo group.

Table 3. Inferential statistics by treatment groups.

	Comparison of Dose groups	Mean	SE	95% Confidence Interval		p-value		Comparison of Dose groups	Mean	SE	95% Confidence Interval		p-value
β -carotene							Vitamin E						
Change 0-3 months	**0mg/day vs. 60 mg/day**	1257.9	85.14	1065.39	1450.41	**<0.0001**	**Change 0-3 months**	**0mg/day vs. 60 mg/day**	0.41	0.44	-0.53	1.35	0.3610
	0mg/day vs. 45 mg/day	1035.78	64.65	888.57	1182.99	**<0.0001**		**0mg/day vs. 45 mg/day**	0.34	0.34	-0.4	1.07	0.3454
	0mg/day vs. 30 mg/day	1109.68	72.56	949.26	1270.1	**<0.0001**		**0mg/day vs. 30 mg/day**	0.77	0.39	-0.05	1.59	0.0633
	0mg/day vs. 15 mg/day	923.02	101.08	697.2	1148.85	**<0.0001**		**0mg/day vs. 15 mg/day**	0.55	0.38	-0.26	1.36	0.1661
Change 0-9 months	**0mg/day vs. 60 mg/day**	1743.71	133.35	1439.83	2047.58	**<0.0001**	**Change 0-9 months**	**0mg/day vs. 60 mg/day**	-1.12	0.45	-2.1	-0.15	**0.0266**
	0mg/day vs. 45 mg/day	1623.84	186.15	1172.3	2075.39	**0.0001**		**0mg/day vs. 45 mg/day**	-1.32	0.42	-2.24	-0.4	**0.0092**
	0mg/day vs. 30 mg/day	1382.17	145.3	1050.47	1713.87	**<0.0001**		**0mg/day vs. 30 mg/day**	-0.67	0.58	-1.96	0.61	0.2743
	0mg/day vs. 15 mg/day	1149.73	197.53	685.68	1613.79	**0.0006**		**0mg/day vs. 15 mg/day**	-0.88	0.39	-1.72	-0.36	**0.0420**

Comment [A36]: Note how much bigger this number was than the analogous number for 3 mos. Suggestive of a tendency for levels to increase over continued administration.

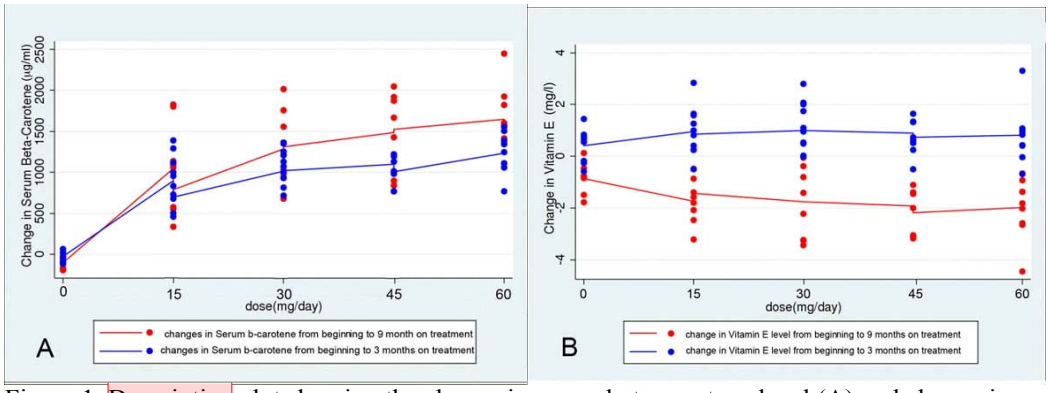

Figure 1. Descriptive plot showing the change in serum beta-carotene level (**A**) and change in vitamin E level (**B**) of different dosage groups. Appended to the figures are lowess curves which illustrate the trend between adjacent dose groups. The blue points are observations of change in level between 3 and 0 months (at randomization), while the red points are observations of the change in level between 9 and 0 months.

Comment [A37]: Jittering would be important to break the ties