Group #15

Study of beta-carotene and vitamin E

Summary

Objective: We aimed to determine how different dose levels of beta-carotene affect the serum beta-carotene levels after 3 and 9 months and to examine whether the administration of beta-carotene affected vitamin E levels in plasma.

Methods: Data analyzed in this study represent a phase II randomized, double-blind trial in which patients (n = 46) were assigned to different dose groups of beta-carotene (15, 30, 45, or 60 mg/day) or to the placebo group for 9 months. Paired t-tests were used to compare serum beta-carotene and vitamin E levels longitudinally within dose groups. Correlations were determined for total average amounts of beta-carotene and vitamin E across dose groups.

Results: We found that administration of beta-carotene at all doses resulted in significant increases in serum beta-carotene levels measured at 3 and 9 months when compared to placebo. These doses of beta-carotene also resulted in a significant increase in time averaged beta-carotene compared to the control group. Administration of 15, 30, 45 mg/day beta-carotene resulted in significant increases in serum vitamin E levels at 3 months. All dose groups and the placebo group showed significant decreases in vitamin E levels at 9 months. No significant differences existed in time averaged vitamin E across dose groups. Time averaged beta-carotene was not correlated with time averaged vitamin E.

Conclusions: All doses resulted in a significant and monotonic increase in serum beta-carotene. We cannot conclude that there is a relationship between beta-carotene dose and vitamin E levels at 3 and 9 months. There is a correlation between beta-carotene dose and total beta-carotene absorbed but not between beta-carotene dose and total vitamin E.

Comment [A1]: It was actually plasma, but I was inconsistent in the documentation.

Comment [A2]: Why? What was the overall goal? (Cancer chemoprevention)

Comment [A3]: This is not a good analysis to address the causative affect of beta carotene supplementation. This anslysi is confounc supplementation with seasonal, aging, and calendar time effects. You need to compare across dose groups, with greatest emphasis on the 15-60 groups vs placebo

Comment [A4]: This analysis did not come from your paired t tests. It could not.

Comment [A5]: Estimates. Estimates. Estimates. Estimates. Estimates. (Re-read everything I have said in this course: statistical significance is not a result by itself. It is a description of the scientific estimates.)

Comment [A6]: Even placebo?

Background

Beta-carotene (provitamin A) and vitamin E (tocopherol) are both fat-soluble antioxidants which have been demonstrated to prevent and slow progression of many diseases several case-control studies. A number of studies support the important role of antioxidants as natural inhibitors which may act on cancer initiation or promotion.Animal studies with supplementation determined that oral doses of beta-carotene and vitamin E in combination reduced tumor size in hamsters (Shklar et al., 1989). The Basel study of 3756 healthy adult volunteers showed a significant inverse correlation between plasma beta-carotene and all cancer, particularly lung and stomach cancer (Stahelin et al., 1989). In the Multiple Risk Factor Intervention Trial, a case control trial of 156 men who died from cancer and 311 matched controls, total beta-carotenoids and beta-carotene levels were lower in the subset of 66 lung cancer cases compared to controls. Although the same study showed no significant differences in beta-carotene levels or in total carotenoids between the group who died from any cancer compared to the control group, the authors concluded beta-carotene may possibly be protective against lung cancer among cigarette smokers (Connett et al., 1989). A case control study of 39 women with breast cancer and 78 matched controls showed that women with breast cancer had significantly lower mean plasma vitamin E levels than women without breast cancer. The authors found a non-significant trend toward lower serum beta-carotene in women with breast cancer compared to women without breast cancer (Wald et al., 1984).

Other studies have not shown a relationship between antioxidants such as beta-carotene and vitamin E and cancer. Russell et al. found no relationship between breast cancer and serum levels

of vitamin E in a nested case control trial of 30 breast cancer cases and 288 age-matched controls (Russell et al., 1988) and, in another case control trial of 22 cases of pancreatic cancer and 44 matched controls, there was not significant association found between serum beta-carotene or vitamin E levels and pancreatic cancer (Burney et al., 1989).

Based on the contradictory findings of these previous studies, a large-scale randomized clinical trial with supplements of beta-carotene and vitamin E, alone or in combination, would be of great interest. However, before such a study can be performed, it is important to understand the pharmacokinetics of beta-carotene and to determine any effects of beta-carotene on vitamin E.

Specific Aims and Study Questions

The specific aims of this study are to: 1) determine how different dose levels of beta-carotene affect serum beta-carotene levels; and 2) to examine whether beta-carotene supplementation is associated with change in vitamin E levels in plasma. The specific questions analyzed were:

1) Does a difference in means exist between serum beta-carotene levels taken at baseline and at 3 and 9 months after randomization within dose groups?
2) Is there an association between dose and mean time averaged beta-carotene?
3) Does a difference in means exist between vitamin E serum levels taken at baseline and at 3 and 9 months after randomization within dose groups?
4) Is there an association between dose and mean time averaged vitamin E?
5) Is there an association between time averaged beta-carotene and time averaged vitamin E?

Comment [A7]: This is NOT the primary question. Instead we want to find out whether beta carotene supplementation is a cause of increased or decreased plasma levels.

Source of Data

Data analyzed are from a double blind randomized phase II prevention trial in which 46 volunteers were randomly assigned to receive one of four doses of beta-carotene (15, 30, 45, or 60 mg/day) or placebo for 9 months. Data included measurements of serum beta-carotene and vitamin E at baseline, at 3 months and at 9 months, and time-averaged beta-carotene and vitamin E. Time average, or the area under the curve (AUC) of a plot of the plasma concentration of drug against time, represents the total amount of drug absorbed by the body regardless of rate of absorption. This measure is useful to determine whether the total amount of beta-carotene absorbed affects the total amount of serum vitamin E, regardless of rate of absorption.
Other data included subjects' age, sex, weight, body mass index (BMI), cholesterol, percent body fat, and time averages of serum levels of beta-carotene and vitamin E. Weight, BMI, cholesterol and percent body fat were used as surrogate measures for subjects' body size and composition.

Comment [A8]: This is definitely true of AUC from single dose studies. But this multiple dose study over quite a protracted period might be better characterized as an AUC representing a time average value.

Unmeasured and Missing Data

Amounts of beta-carotene and vitamin E in participants' diets and fat intake at time of dosage were unmeasured and may potentially have influenced serum levels beyond that of dosing alone. However, randomization theoretically should eliminate any differences that may create a bias between groups. Another concern which arose was that missing data existed in this already small sample size. For example, one subject had baseline serum beta-carotene and vitamin E levels but none at 3 and 9 months. A total of 5 additional subjects, one each in the placebo and the four dose groups, were missing data for serum beta-carotene and vitamin E levels at 9 months. Missing data do not appear to be systematically related to dose, and may therefore be considered non-informative. Finally, this study did not collect data regarding adverse events,

Comment [A9]: Not just theoretically, but actually. Of course, we have to be careful in defining what we mean as bias: conditional or unconditional on the current subjects randomized.

Comment [A10]: We centainly consider it ignorable. It is debatable whether we should or not. There is nothing in our data ever that can tell us whether missing data is truly ignorable.

which limit the ability of the study to detect possible toxicity relating to serum beta-carotene or vitamin E.

Statistical Methods

We performed statistical analysis using Stata 10 software (College Station, TX). A priori, we set the acceptable level of significance as $p \leq 0.05$ and calculated 95% confidence intervals. It is possible that effect modification or precision variables are present in the data, but stratified analysis is likely not useful due to small sample sizes. There are missing data for serum beta-carotene and serum vitamin E at 3 for a single subject, and at 9 months for 6 subjects. Data were analyzed based on intent to treat.

Comment [A11]: This is statistical jargon. Instead say, "some variables could add precision to our analysis if we adjusted for them"

We performed paired t-tests to determine differences between beta-carotene levels at baseline and at 3 months and 9 months within each dose group, accounting for correlated measurements in the data. The same analysis was performed for serum vitamin E levels. These tests answered research questions 1 and 3. For questions 2 and 4 we determined correlations for time average beta-carotene and vitamin E.

Comment [A12]: Which questions were completely irrelevant to our scientific question about the effect of beta carotene supplementation.

To address the question of whether the administration of beta-carotene affects serum vitamin E levels, we determined correlations to assess the association between time average beta-carotene and time average vitamin E (continuous variables).

Results

Characteristics of subjects randomized to dose groups. Within each dose group, distribution of sex, age, weight, BMI, cholesterol and percent body fat were all similar and any differences were not significant (Table 1). We found significant negative correlations indicating lower time averaged beta-carotene in males (r= -0.307, p=0.04) and a strong relationship between weight and time averaged beta-carotene (r=-0.443, p=0.002). Weight and male sex were strongly correlated (r=0.708, p<0.0001). We also found a significant positive correlation between cholesterol level and time averaged vitamin E (r=0.487, p=0.001).

Comment [A13]: Across dose groups

Comment [A14]: What is the importance of these. What correlation is scientifically important? Could this be due to dose?

Comment [A15]: We will not win a Nobel prize for noting this. And correlation is a horrible descriptive statistic in this case—sex is a dichotomous variable. And, besides, correlations CANNOT be sscientifically compared across studies.

Table 1. Descriptive statistics for the sample divided by dose group.

Dose Group (mg/day)	Male (n)	female (n)	Total (n)	Mean age (years)	Mean weight (lb)	Mean BMI (kg/m2)	Mean cholesterol (mg/dl)	Mean percent body fat
0	5	3	8	56.3 ± 4.30	180 ± 32.8	26.6 ± 3.64	218 ± 28.5	28.0 ± 8.2
15	5	5	10	56.3 ± 4.64	168 ± 36.8	25.7 ± 3.58	223 ± 29.7	27.6 ± 8.8
30	3	7	10	57.2 ± 4.08	152 ± 30.2	25.6 ± 2.65	213 ± 33.5	30.3 ± 5.8
45	4	4	8	55.9 ± 3.14	173 ± 40.9	25.3 ± 3.32	213 ± 33.5	32.5 ± 6.0
60	5	5	10	56.5 ± 5.21	159 ± 19.1	24.9 ± 2.43	238.1 ± 38.9	30.4 ± 9.0

Comment [A16]: Presumably mean +/- SD. Say so

Change in serum beta-carotene and serum vitamin E across dose groups. Table 2 presents descriptive statistics on the distribution of serum beta-carotene and vitamin E levels at 0, 3 and 9 months. For all dose groups receiving beta-carotene, serum beta-carotene levels were higher after randomization at both 3 months and at 9 months, when compared to baseline. For all groups including the placebo group, serum vitamin E levels tended to be higher than baseline at 3 months, but tended to be lower than baseline at 9 months. These trends are graphically represented in Figure 1.

Comment [A17]: What about the placebo group. We must know about it, if we are to be able to judge causation here.

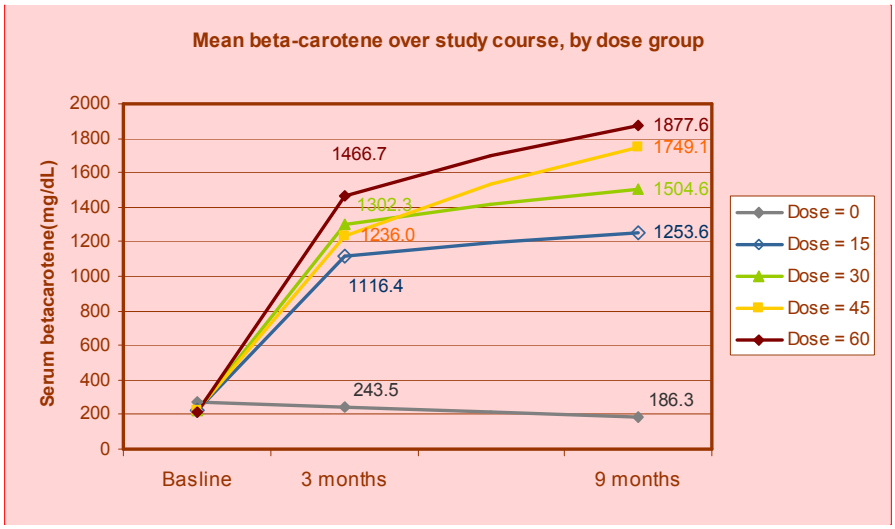
Change in time-averaged beta-carotene and time-averaged vitamin E across dose groups.
We found a significant positive correlation between dose of beta carotene and time averaged beta carotene. The correlation coefficient is 0.7358 ($p<0.0001$). There was no significant correlation between beta-carotene dose and time-averaged vitamin E ($r=0.187$, $p=0.218$).

Comment [A18]: Who cares? What does this correlation mean. I forget the correlation that I try to achieve to prevent cancer.

Table 2. Descriptive Statistics for Serum Beta-Carotene and Vitamin E levels by Dose and Time

Comment [A19]: It is **VERY IMPORTANT** to provide descriptive statistics about the effects of the treatment. You provided mean and CI here. Those are inferential statistics about an average effect. But were any individuals harmed by the treatment? What were the highest and lowest values observed by dose?

		Serum Beta-Carotene Levels by Dose and Time				Serum Vitamin E Levels by Dose and Time			
Time	Dose (mg/day)	Obs	Mean	95% CI		Obs	Mean	95% CI	
0 Months	0	8	270	156	384	8	7.88	6.69	9.06
	15	10	220	129	312	10	7.76	6.89	8.62
	30	10	219	159	279	10	7.98	6.83	9.14
	45	8	227	139	315	8	8.24	7.45	9.04
	60	10	218	130	305	10	8.44	7.53	9.35
3 Months	0	8	244	165	322	8	8.27	7.24	9.3
	15	10	1116	889	1343	10	8.71	8.06	9.36
	30	10	1302	1116	1488	10	9.15	8.51	9.8
	45	8	1236	1036	1436	8	8.98	8.45	9.51
	60	9	1467	1274	1660	9	9.11	8.61	9.62
9 Months	0	7	186	105	268	7	7.25	6.21	8.29
	15	8	1254	777	1731	8	5.75	5.33	6.17
	30	9	1505	1136	1873	9	6.3	5.42	7.17
	45	7	1749	1214	2285	7	6.15	5.34	6.96
	60	9	1878	1547	2208	9	6.32	5.46	7.17
Time Average (AUC)	0	8	234	158	311	8	7.79	6.86	8.73
	15	10	1132	903	1361	10	7.97	7.36	8.58
	30	10	1337	1142	1531	10	8.36	7.55	9.16
	45	8	1324	1076	1573	8	8.04	7.63	8.44
	60	9	1523	1331	1714	9	8.35	7.79	8.92



Comment [A20]: It would be nice to see data for individuals to be able to judge toxicity.

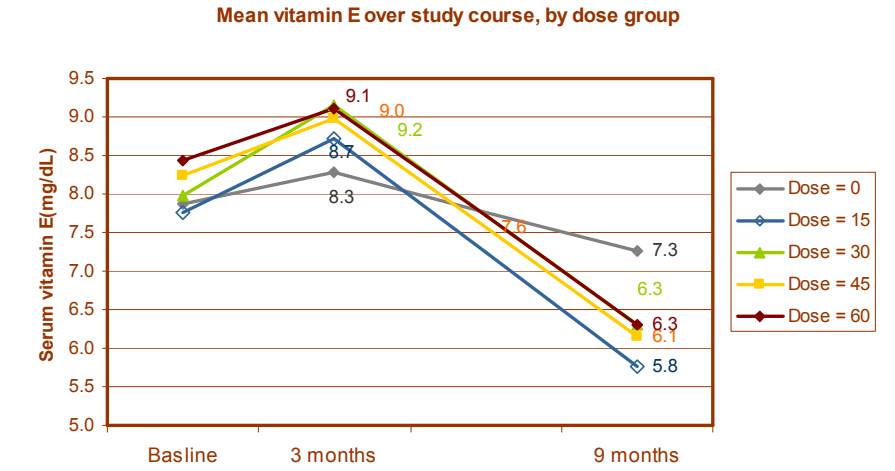


Figure 1. Patient serum beta-carotene and vitamin E levels over study time.

In examining mean differences in serum beta-carotene levels over time within all dose groups (excluding placebo), we found that there is a trend towards dramatically increasing serum beta-carotene level from 0 to 3 months and, at a less striking rate, from 3 to 9 months. In the placebo group, levels tended to decrease, though this was only significant from 0 to 9 months ($p = 0.007$) and from 3 to 9 months ($p = 0.009$). These findings are summarized in Table 3. Mean serum beta-carotene levels also increased significantly from time of randomization to 9 months in all dose groups.

Comment [A21]: Scientific relevance?

Significant changes at 3 and 9 months after baseline were also observed in each dose group for serum vitamin E (Table 3). Mean serum vitamin E significantly increased from time of randomization to 3 months for those subjects receiving 15, 30, and 45 mg/day beta-carotene. The placebo group and the 60 mg/day group also showed increases of 0.398 (95% CI -0.169,

0.965) and 0.811 (95% CI -0.031, 1.654) respectively, but these were not significant (p = 0.141, p = 0.057 respectively). All dose groups including the control group showed highly significant decreases in vitamin E levels from 0 to 9 months.

Comment [A22]: scientific relevance?

Table 3: Change in beta-carotene and vitamin E levels during course in study (assessed by paired t-test). Significant p values in bold.

		N	Mean diff.	95% CI		p-value†
Beta-carotene						
0 vs. 3 months						
	0	8	-26.7	-76.4	22.9	0.244
	15	10	896	673	1120	**<0.001**
	30	10	1083	926	1240	**<0.001**
	45	8	1009	864	1154	**<0.001**
	60	9	1231	10411	1421	**<0.001**
0 vs. 9 months						
	0	7	-102	-163	-39.8	**0.007**
	15	8	1048	585	1511	**0.001**
	30	9	1281	951	1611	**<0.001**
	45	7	1522	1071	1974	**<0.001**
	60	9	1642	1340	1944	**<0.001**
Vitamin E						
0 vs. 3 months						
	0	8	0.398	-0.169	0.965	0.141
	15	10	0.951	0.281	1.622	**0.011**
	30	10	1.168	0.485	1.85	**0.004**
	45	8	0.733	0.152	1.314	**0.020**
	60	9	0.811	-0.031	1.654	0.057
0 vs. 9 months						
	0	7	-0.863	-1.445	-0.282	**0.011**
	15	8	-1.742	-2.469	-1.014	**0.001**
	30	9	-1.535	-2.763	-0.306	**0.021**
	45	7	-2.184	-3.02	-1.347	**0.001**
	60	9	-1.988	-2.868	-1.108	**0.001**

† Two-sided p value

Time-averaged beta-carotene and vitamin E. We found a significant positive correlation between dose of beta-carotene and time averaged beta-carotene. The correlation coefficient was quite high: 0.7358 (p<0.0001). There was no significant correlation between dose and time-averaged vitamin E (r=0.187, p=0.218).

Comment [A23]: What does this number mean?

Discussion

For all doses of beta-carotene there was a monotonic increase in serum beta-carotene levels from baseline to 9 months, whereas the placebo group did not show a significant increase over the same time period. Although we used a multiple comparison method, which may increase the possibility of Type I error, our results were highly significant. The effect on serum beta-carotene was rapid and sustained over the 9 month period.

Comment [A24]: Again, this is not the comparison that answers our scientific question.

Although we found that weight and male sex are negatively correlated with time-averaged beta-carotene, we have no reason to believe that sex has a differential effect on time averaged beta-carotene. The observed association may be largely explained by weight as male sex is positively correlated with higher weight in this sample. We note that beta-carotene doses were not adjusted for weight and that lighter subjects may receive a relatively higher amount of beta-carotene despite receiving the same dose as heavier subjects. Interestingly, body mass index, which is weight in kilograms divided by height in meters squared, is not significantly correlated with time averaged beta-carotene (p=0.101). This suggests that when weight is adjusted for height, the association is no longer significant. Due to the small sample size, the ability to perform adjusted analyses for these associations is relation to serum beta-carotene and vitamin E is severely limited.

We find that mean serum vitamin E levels tend to increase at 3 months for all dose groups, but found statistical significance in only the 15, 30 and 45 mg/day dose groups. Moreover, we see a highly significant decrease in mean serum vitamin E levels in all groups including the control group at 9 months, which may be an observation related to unmeasured confounding variables. We also do not find any significant correlation between the total amount of beta-carotene absorbed (time averaged beta-carotene) and the total amount of vitamin E (time averaged vitamin E). The longitudinal response of serum vitamin E is inconsistent with a correlated response with beta-carotene dose. From our results, we cannot conclude that there is a relationship between the administered beta-carotene dose and vitamin E levels.

The findings of this study suggest several directions for further research. This trial is limited by small sample size which restricts ability to perform adjusted analyses to address the correlations observed for patient characteristics with outcome beta-carotene and vitamin E levels. The trial also does not adequately explain the significant decrease in vitamin E levels at 9 months compared to baseline, nor is it able to provide any information on potential toxicity or adverse events associated with beta-carotene dose. In addition, although no significant dose-response relationship was observed at 15 mg/day increments, there did appear to be a fairly consistent relationship between dose and 3- and 9-month serum beta-carotene levels, such that higher dose tended to be associated with higher beta-carotene level. The lack of statistical significance may be due to low statistical power relating to inadequate sample size. A larger sample size would therefore help to clarify whether these trends are true, or merely were observed by chance.

Research more specifically assessing the link between beta-carotene association and clinical disease is critical in evaluating whether to practice preventive supplementation. Finally, the nine-month follow-up time may not be sufficiently long to evaluate the longer-term effects—either protective or adverse—of beta-carotene supplementation; longer-term monitoring of patients taking beta-carotene supplements is therefore recommended.

Comment [A25]: All of this analysis is irrelevant. How did you adjust for dose when doing these correlations? I am worried that you believe that correlation is telling you anything here. It is of little value beyond the p value. In fact, if you tell me the p value and the sample size, I can pretty much tell you the correlation.

Comment [A26]: and the scientific relevance is…

References

Connett JE, Kuller LH, Kjelsberg MO, Polk BF, Collins G, Rider A and Hulley SB (1989) Relationship between carotenoids and cancer. The Multiple Risk Factor Trial Study. *Cancer* 64(1): 126-134.

Burney PG, Comstock GW and Morris JS (1989) Serologic precursors of cancer: serum micronutrients and the subsequent risk of pancreatic cancer. *Am J Clin Nutr* 49(5):895-900.

Russell MJ, Thomas BS and Bulbrook RD. (1988). A prospective-study of the relationship between serum vitamin A and vitamin E, and risk of breast cancer. *Br J Cancer* 57(2):213-215.

Stahelin HB, Gey F and Brubacher G. (1989) Preventive potential of antioxidative vitamins and carotenoids on cancer. *Int J Vitam Nutr Res Suppl* 30: 232-241.

Shklar G, Schwartz J, Trickler D and Reid S (1989) Regression on experimental cancer by oral administration of combined alpha-tocopherol and beta-carotene. *Nutr Cancer* 12(4): 321-325.

Wald NJ, Nicolaidesbourman A and Hudson GA. (1988) Plasma retinol, beta-carotene and vitamin E levels in relation to the future risk of breast-cancer. *Br J Cancer* 57(2): 235-235.