SERUM RESPONSE TO BETA-CAROTENE SUPPLEMENTATION; A PHASE II TRIAL
Group 7

Summary

Background: Beta-carotene is a fat-soluble antioxidant carotenoid that may prevent cellular damage and may have a beneficial impact on a variety of diseases. Beta-carotene is thought to be a benign substance, but may influence absorption of other fat soluble vitamins. Prior studies have assessed serum response to low doses (15 mg/day) of beta carotene, but we are not aware of any studies that have evaluated the impact of higher doses.

Objective**:** To evaluate the pharmacokinetic characteristics of beta-carotene supplementation over time, and to examine the effect of beta-carotene on serum vitamin E levels.

Methods**:** Forty-six volunteers were randomly assigned to receive one of five doses of beta-carotene (0, 15, 30, 45, or 60 mg/day) for 9 months. Serum beta-carotene and serum vitamin E concentrations were measured prior to treatment and after 3 and 9 months of continual treatment. Forty-five subjects provided at least one follow-up serum sample, and serum concentrations for these patients were compared across dose levels and over time. Baseline measurements included BMI, body fat percentage, weight, serum cholesterol, and age.

Results**:** The estimated dose effects differ significantly between beta-carotene supplementation groups. The greatest increase in serum beta-carotene concentration was seen in the first 3 months with the 15 mg/day group showing a mean serum beta-carotene level 873 points higher than placebo (95% CI: 640 to1106, $p<0.001$) and 896 points greater than at baseline (95% CI: 673 to 1120, $p<0.01$). Between 3 and 9 months in the 15 mg/day group serum levels increased 172 points (95% CI: 46 to 391, $p=0.1$). Vitamin E levels decreased between 3 and 9 months in all treatment groups. In the 15 mg/day beta-carotene group vitamin E levels dropped 2.97 points (95% CI: 3.4 to -2.3, $p<0.001$), and in the 60 mg/day beta-carotene group levels dropped 2.80 points (95% CI: 3.5 to -2.1, $p<0.001$).

Conclusions**:** The primary effects of beta-carotene supplementation are observed in patients taking 15 mg/day, and occur within the first three months of treatment. Serum concentrations "level off" and increase moderately with dose when comparing the 15 mg/day group to other groups with higher doses, and increase slightly through the time period between 3 months and 9 months after randomization. Vitamin E concentrations drop significantly below normal after nine months of beta-carotene supplementation.

Comment [A1]: What was the specific goal here? (Ultimately cancer chemoprevention.)

Comment [A2]: Actually, it was plasma. But I did have it mislabeled in the definition of the variables. I will have to fix that.

Comment [A3]: I would also have talked about the dose 60 group at least.

Comment [A4]: The major comparison should be between dose groups, not over time, because the latter is not randomized.

Comment [A5]: This wording is a little misleading, I think. I suppose you were meaning that there was little additional increase due to doses above 15 mg. But you should say that and quantify it.

Comment [A6]: I'll take your word for it, but I don't know the normal range, personally.

Background

Beta-carotene is a fat soluble anti-oxidant carotenoid that may prevent cellular damage, and consequently may have a beneficial impact on the treatment and prevention of cancer, heart disease, macular degeneration, and cognitive decline.

Early studies [1, 2] demonstrated that smokers with lung cancer reported eating less green leafy and orange vegetables than those smokers who did not develop lung cancer. These studies called for randomized controlled trials to further examine such an association. Investigation of mouse models strengthened the hypothesis that there is anti-tumor activity of beta-carotene[3]. In-vitro studies also showed that beta-carotene alone or in conjunction with vitamin E can decrease the incidence of vascular events[4].

Later studies examined the potential protective relationship of beta-carotene on cancers. The British United Provident Association study showed an association between beta-carotene intake and a decreased risk of developing lung cancer[9]. The Physician's Health Study was undertaken to prospectively examine the association between beta-carotene and cancer risk[10]. By 1990, the results of this study had not been published.

The anti-oxidant property of beta-carotene is mediated through a novel mechanism of radical trapping[5]. Beta-carotene is a pro-vitamin of vitamin A, and therefore causes increased serum vitamin A levels. Additionally, high doses of beta-carotene may also influence concentrations of other fat soluble vitamins such as vitamin E, vitamin K, and vitamin D. The association between beta-carotene and vitamin E has been examined. Studies prior to 1990 showed no effect of beta-carotene supplementation on serum vitamin E levels[6] [7]. However, high doses of vitamin E were shown to reduce serum and hepatic levels of beta-carotene in rats[8], so it is plausible that high doses of beta-carotene could reduce serum and hepatic levels of vitamin E to harmful levels.

A pilot study of the pharmacokinetic properties of beta-carotene supplementation showed that beta-carotene levels increased 10-fold over 4 months in men treated with 15 mg of beta-carotene orally. Analysis included relationships between BMI, alcohol consumption, smoking and lipids. No toxicities or skin changes were observed[11]. In this study, we analyze the pharmacokinetics of different doses of beta-carotene supplementation and its relationship to vitamin E levels in men and women of varying body compositions.

Questions of Interest

The primary question of interest is how various dosage levels of daily beta-carotene supplementation affect serum beta-carotene levels 3 months and 9 months after beginning supplementation. The secondary question of interest is the dose effect of beta-carotene supplementation on plasma Vitamin E levels.

Comment [A7]: Given the primary question stated in this way, I certainly would have reported at least the dose 15 and dose 60 groups in the abstract.

Materials and Methods

Study Design: We conducted a double-blind, randomized, placebo controlled Phase II clinical trial to evaluate the pharmacokinetic properties of beta-carotene supplementation. Forty-six volunteers gave informed consent and were randomized at baseline to treatment groups defined by dosage of beta-carotene (0, 15, 30, 45, or 60 mg/day). Treatment doses were administered throughout the 9-month study period. A total of 45 subjects completed the Phase II clinical trial. One enrolled subject was dropped from analysis for lack of follow-up measurements, leaving 8, 10, 10, 8 and 9 subjects in the 0-60 mg/day dose groups, respectively.

Baseline demographic characteristics were collected, and serum chemistry, beta-carotene and vitamin E levels were measured. Subjects were followed for 9 months after randomization. Beta-carotene and vitamin E plasma concentrations were measured at 3 months and 9 months post-randomization. This study protocol was approved by the Institutional Review Board.

Comment [A8]: at least you hope it was. (I guess I didn't put that in the description.)

Data Sources: Baseline demographic and serum chemistry characteristics for all 45 participants were collected and compared across dosage groups to assess balance across treatment groups. Demographic characteristics included age, weight, BMI, cholesterol and body fat percentage. Plasma concentrations included levels of beta-carotene and vitamin E. As a way to assess overall serum concentrations during the entire study period, time-weighted concentrations were computed using the standard "area under the curve" (AUC) estimation. The overall value is computed by multiplying the mean concentrations in each time interval by the length of the interval, summing across time intervals, and dividing by the total length of the study.

Comment [A9]: And in case you care, monthly measurements were actually used for this.

Statistical Analysis: Five patients participated only through the three-month evaluation and then dropped out. All five patients who dropped out after three months were women. Serum beta-carotene and vitamin E values in these five patients were similar to values in the remaining female subjects, and dropout was spread fairly evenly across dose groups. (There was one dropout in each of the 0, 30 and 45 mg/day groups, 2 dropouts in the 15 mg/day group, and no dropouts in the 60 mg/day group.) Therefore, there was no evidence to suggest that treatment

Comment [A10]: I would probably report this as a result, because it is possible that treatment was the cause of dropout. But in an observational study, I am more prone to discuss such things in materials and methods.

influenced dropout. Three-month data for the patients who dropped out at 9 months was included in the analysis. The degree to which patients adhered to the daily treatment regimen is unknown.

Serum concentrations were compared between different dose groups using unpaired t-tests, and changes over time within the same dose groups were compared using paired t-tests. Equal variances were not assumed in any test. No advanced modeling was performed, and no adjustments were made to account for multiple comparisons. Due to the large number of statistical tests performed in this analysis, all p-values should be interpreted with caution. For subgroups defined by sex, weight, BMI, percent body fat, and cholesterol, exploratory analyses were performed using similar pair-wise tests. Statistical analyses were performed using R version 2.5.0.

Comment [A11]: I probably would have identified some "primary" comparison.

Comment [A12]: I would not have bothered with these: We have a placebo group. Any causative effect is tied up with the comparison across dose groups.

Comment [A13]: good to note

Results

Patient characteristics

Among the 45 patients included in the analysis, 21 (47%) were male and the mean age was 56 years old (range: 50 to 64). The mean weight was 75.0 kg (range: 53.6 to 115.0), the mean body mass index (BMI) was 25.5 (range: 20 to 32), the mean percent body fat was 29.7% (range: 16% to 44%), and the mean cholesterol level was 220 mg/dl (range: 159 to 312). As a result of randomization, all treatment groups were reasonably well balanced with respect to these variables (Table 1).

Baseline Serum Beta Carotene and Vitamin E

At the time of baseline laboratory assessment, the mean serum beta-carotene was 233 (range: 48 to 496) and was similar across treatment groups (Table 2) and similar for males and females. The mean serum vitamin E concentration at baseline was 8.0 mg/dl (range: 5.1 to 10.7) and also was similar across treatment groups and similar for males and females. None of the subjects had serum levels that would be considered outside the normal range.

3 and 9 Month Follow-up Serum Beta-carotene and Vitamin E:

At 3 and 9 months from baseline, subjects who received placebo tended to have slightly lower serum levels of beta-carotene compared to baseline, with the mean beta-carotene serum level dropping from 270.2 to 186.3 mg/dl. Subjects who received supplemental doses of beta-carotene had higher serum levels at both 3 and 9 months, with larger doses tending to yield higher beta-carotene serum levels (Table 2, Figure 1). Beta-carotene serum concentrations at 3 months ranged from a mean of 1116 mg/dl for patients receiving the 15 mg/day dose, to a mean of 1467 mg/dl for patients receiving the 60 mg/day dose. 9 month beta-carotene serum levels ranged from 1254 mg/dl for subjects receiving 15 mg/day, and to 1878 mg/dl for subjects receiving 60 mg/day.

Comment [A14]: What do you make of this observation? Diet is the likely culprit, but laboratory drift could be a problem. In any case, I would not play it up too much, instead focusing on comparisons across dose groups.

Comment [A15]: It is important to again note that you are talking about the mean values here. We would also be interested in the max values for each group, because that might be an indication of toxicity, and we need to distinguish which is which.

For all patients except those randomized to receive placebo, the variability in beta-carotene serum concentration increased markedly at each time interval. For patients taking 15 mg/day, beta-carotene serum levels at baseline ranged from 65 to 496 mg/dl (SD=40), while at three months the range increased to 699 to 1603 mg/dl (SD=100), and at 9 months the range increased again to 577 to 2019 mg/dl (SD=202). Similar trends are evident for each dose group (Table 2, Figure 1 (A)), with beta-carotene serum-level standard deviations approximately doubling at each observation time.

Comment [A16]: Very good to note range of measurements within each group: The reader might be worried about toxic levels.

Vitamin E levels were slightly higher at 3 months as compared to baseline, but the mean concentrations were very similar across beta-carotene dose groups. Almost no change was observed in the placebo group. Contrary to the pattern observed in beta-carotene levels, the variation in vitamin E level tended to decrease as indicated by measurements taken at 3 months (Table 2, Figure 1 (B)). For example, among subjects randomized to 60 mg/day the standard deviation of vitamin E level fell from 1.27 to 0.66 mg/dl during the first 3 months.

Comment [A17]: Mainly of interest to me in my never-ending quest to get everyone to appreciate the need for placebo groups.

Comment [A18]: Well, this could be similar to the fact that there was a mean-variance relationship in which groups with higher means had higher variation.

At 9 months, vitamin E levels dropped noticeably for all subjects receiving at least 15 mg/day of beta-carotene. Again there was very little difference in vitamin E levels according to treatment group, but in all cases the mean level dropped well below the baseline measurements. For instance, the 15 mg/day group dropped from 8.71 to 5.75 mg/dl, and the 60 mg/day group dropped from 9.11 to 6.32 mg/dl. At 9 months the variation in vitamin E level returned to baseline levels for most groups (Table 2, Figure 1 (B)).

Comment [A19]: In the abstract, you claimed they droppedto below normal levels. You should comment on that here.

Trends and Statistical Significance

The most pronounced differences in serum beta-carotene levels occurred between the 0 and 15 mg/day dose groups. At three months, subjects receiving 15 mg/day had serum beta-carotene levels 873 mg/dl higher than subjects in the 0 mg/day group (95% CI: 640 to 1106), and this difference was highly significant with $p<0.001$ (Table 3). Each additional 15 mg/day in dosage yielded relatively small, statistically insignificant incremental changes. Taken together, however, the difference between 15 and 60 mg/day was 350 mg/dl (95% CI: 74 to 626), which was significant at $p=0.016$. Similar trends were observed at 9 months (Table 3).

Comment [A20]: Nicely stated.

For all dose groups, most of the increase in beta-carotene levels occurred within the first 3 months of treatment, with smaller increases occurring from 3 to 9 months (Table 3). Mean beta-carotene levels for subjects in the 15 mg/day increased by 896 mg/dl in the first 3 months (95% CI: 673 to 1120, $p<0.001$), but increased by only 172 mg/dl points during the subsequent 6 months (95% CI: 46 to 391, $p=0.1$). The higher dose groups had larger increases during both time periods, for example the 60 mg/day group increased by 1231 mg/dl during the first 3 months on treatment (95% CI: 1041 to 1421, $p<0.001$), and an additional 411 mg/dl from 3 to 9 months (95% CI: 156 to 666, $p=0.006$).

Comment [A21]: Again, nicely stated

While treatment dose had relatively small, statistically insignificant effects on serum vitamin E, changes over time were notable in all groups with beta-carotene doses larger than 15 mg/day. In most treatment groups, serum vitamin E increased by a modest but statistically significant amount during the first 3 months (Table 3). However, between 3 and 9 months all groups experienced dramatic drops in serum vitamin E, all highly significant with $p<0.001$.

Comment [A22]: All. Including placebo. So what do we make of it? (Probably diet or laboratory drift)

Comment [A23]: We care about comparisons between dose groups, more than we care about changes within dose group over time.

Exploring body composition and serum beta-carotene

Weight and percent body fat were closely related to baseline beta-carotene levels, and apparently affected absorption rates. The most prominent effect modifier was sex, with females reaching higher serum beta-carotene levels at lower treatment doses than men. For instance, at 9 months, serum levels were 960 mg/dl higher for women than men at the 15 mg/dose (95% CI: 468 to 1453, $p=0.003$). However, serum levels tended to converge at higher doses, with both sexes having similar serum levels at the 60 mg/day dose.

Discussion

In this Phase II trial, we sought to characterize the pharmacokinetic properties of beta-carotene over time. We also examined the relationship of beta-carotene dose and serum vitamin E levels. In 9 months of follow-up, we observed that serum beta-carotene levels increased in all supplementation groups, and that vitamin E levels showed a modest increase at 3 months, but a large decrease at 9 months across all treatment groups.

Comment [A24]: This statement does not help. We care whether the effects in the supplemented groups differ from the placebo group. You should tell us whether that happened. We had the placebo group precisely because we were afraid there might be differences over time.

Our study found significant increases in serum beta-carotene in all treatment groups, with the greatest increases occurring in the first 3 months of supplementation. A 5-fold increase in beta-carotene serum levels was observed at the 3-month follow-up for the 15 mg/day dose group. This increase was less than that found by Constantino *et al.*[11], who found a 10-fold change in beta-carotene serum levels at a 4 month follow up in patients receiving a 15 mg beta-carotene supplementation. In general, there was an approximately 5-fold increase in all dose groups from

Comment [A25]: Very nice to compare your results to previous literature. Were the baseline levels also similar? Was there possibly a seasonal effect? Or were these differences perchance consistent with random sampling error? Or could we tell?

baseline to 3 months, while a 6 to 8 fold increase was observed in all dose groups at the 9 month follow-up.

The results of this investigation found gender to be an effect modifier with respect to serum beta-carotene levels in the lower beta carotene dosage groups. There was a trend to convergence in the higher beta-carotene dosages (45 and 60 mg/dl). Previous studies have not revealed a gender effect in beta-carotene serum levels. Although we did not have sufficient data to perform a detailed subgroup analysis, gender effects should be explored in further investigations.

Based on these results, we are unable to determine whether beta-carotene supplementation will a have negative physiologic impact on the absorption other fat soluble vitamins. The results of this study show that increasing doses of beta-carotene tend to decrease serum Vitamin E levels, possibly by decreasing intestinal transport or hepatic storage. In general, we would expect the serum levels of other fat soluble vitamins to steadily decrease with increasing beta-carotene intake and absorption. The long-term consequences of this process are unknown. In this study, vitamin E levels were at the threshold of low-normal. Serum vitamin E levels below 5.0 mg/l indicate deficiency. In this study, mean serum vitamin E levels did not go below 5.26 mg/l. However, low serum levels of vitamin E have been associated with neuromuscular dysfunction, so it is, therefore, important to further investigate this trend.

Comment [A26]: And as a footnote, these results (and my including it as a question) had a data-driven aspect to it. These results have not been replicated in far larger studies. Just goes to show the bad effects of data-driven analyses.

Numerous physiological features may influence the degree to which specific doses of beta-carotene affect actual serum concentration. Our study found that weight, BMI, and percent body fat all affected the dose-response relationship. The most notable difference was observed between males and females, where serum beta-carotene in females tended to be higher than for males in the same dose group. Further study is needed to evaluate how dosages should be adjusted for different patients of varying sizes, composition, and gender.

Limitations

Adherence to the treatment protocol was not measured and may be a critical factor in explaining increased variation in serum concentration over time. If some subjects failed to take the supplements regularly during the later months of the study, we might expect the variation in serum levels to be higher at nine months. Variables such as weight, BMI, percent body fat, and gender were all identified as potentially influencing the dose at which a specific serum concentration is achieved. However, this study was not designed to assess the effects of those features and further study would be needed to fully quantify these. Another limitation is that we did not measure dietary intake among these subjects. Variation in dietary habits may to some extent explain differences in serum levels, and some subjects may modify eating habits as a result of beta-carotene supplementation.

Comment [A27]: maybe they didn't lie the orange hue they were taking on.

Comment [A28]: I actually use just the converse to try to explain the difference between the observational data suggesting beta carotene in your diet is good and the RCT data suggesting beta carotene supplementation is bad.

Conclusions

The results of this Phase II clinical trial provide evidence that the dose effect of increased beta-carotene persists over time, while serum vitamin E decreases in the period between 3 and 9 months. We are unable to explain why serum beta-carotene levels become more variable as time goes on. It may be that individuals metabolize beta-carotene differently or that there was a broad range of treatment adherence. The current study suggests that supplemental beta-carotene can be safely given. We would recommend monitoring serum vitamin E levels in all patients receiving beta-carotene supplementation longer than 3 months duration. The lack of attrition indicates that the treatment regimen was reasonable and that toxicity and adverse events were negligible. It is therefore reasonable to advance beta-carotene studies to Phase III, multi-center clinical trials in order to assess clinical importance in prevention of cardiovascular disease and cancer.

Comment [A29]: I would state things relative to randomization rather than the 3 monthe to 9 months period. But acceptable to me would be to first give the baseline to 9 monthsand then contrast what had happened by 3 months.

Comment [A30]: mean-variance relationships are quite common

References:

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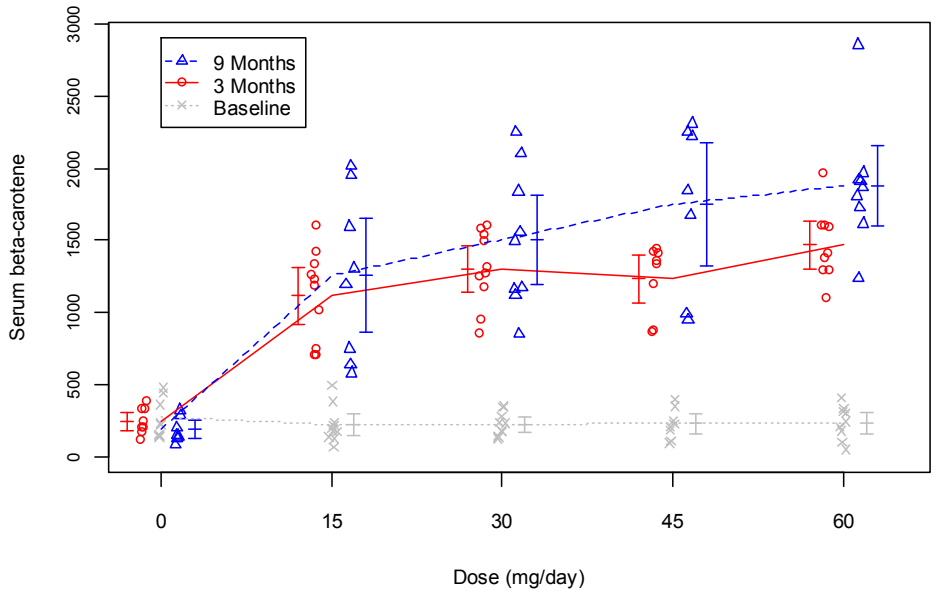
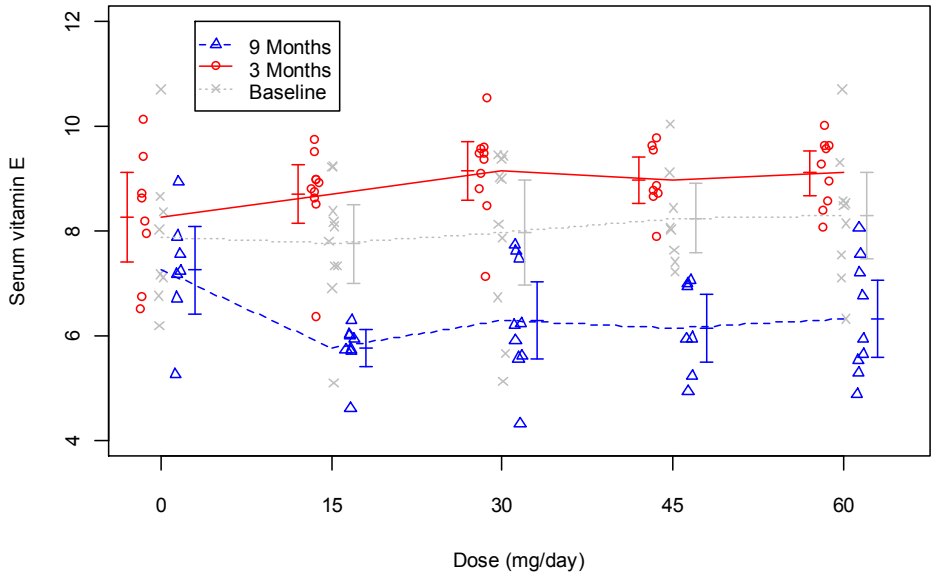
11. Costantino, J., et al., *Serum level changes after administration of pharmacologic doses of beta-carotene.* American Journal of Clinical Nutrition, 1988. **48**(5): p. 1277-1283.

Figure 1. **(A)** Serum beta-carotene at baseline, three months, and nine months according to dosage group. **(B)** Serum vitamin E at baseline, three months, and nine months according to dosage group. Plotted lines connect the sample means, and error bars are 95% confidence intervals centered at the mean. (For the sake of readability, points and lines are slightly offset from the actual dose.)

(A)



(B)



Comment [A31]: Within each dose group, I would have put time in order here: baseline then 3 month then 9 month, instead of 3 month then baseline then 9 month.

Table 1. Summary statistics at randomization by dose group

	0 (n=8) *63% males*		15 (n=10) *50% males*		30 (n=10) *30% males*		45 (n=8) *50% males*		60 (n=9)* *48% males*	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age (yrs)	56.2	4.3	56.3	4.64	57.2	4.08	55.9	3.14	56.5	5.21
Weight(kg)	81.0	14.9	76.3	16.7	69.1	13.7	78.5	18.5	72.3	8.7
BMI (kg/m^2)	26.5	3.64	25.7	3.58	25.6	2.65	25.3	3.32	24.9	2.43
Chol. (mg/dl)	218	28.5	223	29.7	213	33.5	213.3	33.5	238	38.9
Fat %	0.28	0.08	0.28**	0.09	0.3	0.06	0.32	0.06	0.3	0.09
Beta-carotene (mg/dl)	270	136	220	129	219	83.8	227	106	218	122
Vit. E (mg/dl)	7.88	1.42	7.76	1.21	7.98	1.62	8.24	0.95	8.44	1.27

* One male dropped from 60 mg/day dose group because of absent post-randomization measurements

** One value is missing for fat percentage in the 15 mg/day group

Table 2. Serum beta-carotene and vitamin E levels by beta-carotene supplemental dose group

	Beta Carotene Treatment (mg/day)									
	0 (n=8)		15 (n=10)		30 (n=10)		45 (n=8)		60 (n=9)	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Serum Beta carotene (mg/dl)										
Baseline	270 (48.2)	136 476	220 (40.5)	64.8 496	219 (26.5)	125 348	227 (37.3)	93.3 395	235 (115)	48.3 407
3 Months	243 (33.4)	109 384	1116 (100)	699 1603	1302 (82.2)	854 1603	1236 (84.6)	860 1440	1467 (251)	1098 1960
9 Months	186 (33.2)	84.5 323	1254 (202)	577 2019	1505 (160)	849 2248	1749 (218)	950 2310	1878 (430)	1233 2855
Serum Vitamin E (mg/dl)										
Baseline	7.88 (.50)	6.19 10.71	7.76 (.38)	5.10 9.24	7.98 (.51)	5.12 9.46	8.24 (.33)	7.22 10.0	8.30 (1.27)	6.32 10.7
3 Months	8.27 (.44)	6.50 10.11	8.71 (.29)	6.36 9.74	9.15 (.28)	7.12 10.55	8.98 (.22)	7.89 9.78	9.11 (.66)	8.07 10.0
9 Months	7.25 (.43)	5.26 8.93	5.75 (.18)	4.61 6.28	6.30 (.38)	4.31 7.74	6.15 (.33)	4.94 7.05	6.32 (1.12)	4.87 8.06

Table 3. Mean differences in serum beta carotene and vitamin-E levels by dose (mg/day) and time interval, 95% confidence intervals, and p-values*.

Differences in mean serum concentration for incremental increases in dose

	Dose 0 vs 15			Dose 15 vs 30			Dose 30 vs 45			Dose 45 vs 60			Dose 15 vs 60		
	difference	**95% C.I.**	**p-value**	**difference**	**95% C.I.**	**p-value**	**difference**	**95% C.I.**	**p-value**	**difference**	**95% C.I.**	**p-value**	**difference**	**95% C.I.**	**p-value**
Serum Beta Carotene (mg/dl)															
3 months	873	640, 1106	<0.001	186	-87, 459	0.17	-66.3	-317, 184	0.58	231	-23, 484	0.072	350	74, 626	0.016
9 months	1067	589, 1546	0.001	251	-302, 804	0.35	244	-348, 837	0.39	128	-449, 706	0.63	624	89, 1159	0.026
Overall**	897	663, 1132	<0.001	205	-75, 484	0.14	-12.4	-303, 278	0.93	198	-90, 486	0.16	391	114, 667	0.008
Serum Vitamin E (mg/dl)															
3 months	0.44	-0.7, 1.6	0.42	0.44	-0.4, 1.3	0.29	-0.17	-0.9, 0.6	0.64	0.14	-0.5, 0.8	0.67	0.41	-0.4, 1.2	0.28
9 months	-1.50	-2.6, -0.4	0.011	0.54	-0.4, 1.5	0.22	-0.15	-1.2, 0.9	0.77	0.17	-0.9, 1.2	0.74	0.56	-0.3, 1.5	0.20
Overall**	0.18	-0.9, 1.2	0.72	0.39	-0.6, 1.3	0.40	-0.32	-1.2, 0.5	0.43	0.32	-0.3, 1.0	0.31	0.38	-0.4, 1.2	0.31

Change in mean serum concentration over time, from 0 to 3 months and 3 to 9 months

	Dose 0			Dose 15			Dose 30			Dose 45			Dose 60		
	change	**95% C.I.**	**p-value**	**change**	**95% C.I.**	**p-value**	**change**	**95% C.I.**	**p-value**	**change**	**95% C.I.**	**p-value**	**change**	**95% C.I.**	**p-value**
Serum Beta Carotene (mg/dl)															
0-3 months	-26.7	-76, 23	0.24	896	673, 1120	<0.001	1083	926, 1240	<0.001	1009	864, 1154	<0.001	1231	1041, 1421	<0.001
3-9 months	-68.4	-112, -24	0.009	172	-46, 391	0.10	229	-43, 500	0.088	530	187, 874	0.009	411	156, 666	0.006
Serum Vitamin E (mg/dl)															
0-3 months	0.40	-0.2, 1.0	0.14	0.95	0.3, 1.6	0.011	1.17	0.5, 1.8	0.004	0.73	0.2, 1.3	0.02	0.81	0.0, 1.7	0.057
3-9 months	-1.24Thi	-1.7, -0.8	<0.001	-2.87	-3.4, -2.3	<0.001	-2.83	-3.6, -2.1	<0.001	-2.99	-3.7, -2.3	<0.001	-2.80	-3.5, -2.1	<0.0

Comment [A32]: This difference highlights the importance of a placebo group.

* All p-values are derived from two-sided t-tests assuming unequal vaiances.

** Time-weighted serum levels ("area under the curve").