Group 11
Biostat 517
Group Project

Abstract

Background: Cancer is responsible for a huge burden of morbidity and mortality around the world. Recent epidemiologic studies have associated cancer with lower β-carotene levels in humans. However, the inference of causation from these studies needs to be strengthened by evidence from randomized, controlled trials of β-carotene supplementation. Prior to commencement of such a trial, more needs to be known about the pharmacokinetics of β-carotene in healthy adults, specifically dose-response, and any adverse effect on vitamin E levels.

Materials & Methods: As part of a phase II prevention trial 46 adult volunteers were randomized to receive 0, 15, 30, 45, or 60 mg/day of β-carotene for nine months in a double blind fashion. Serum levels of β-carotene and vitamin E were measured at baseline, 3 months and 9 months.

Results: There was a statistically significant increase in serum β-carotene between baseline and 3 months in all treatment groups with the effect ranging from a mean ratio (3 month:baseline) of 6.45 (95% CI 4.02 to 8.88, p=0.0007) in the 15 mg/day group to 9.10 (95% CI 3.21 to 14.99, p=0.0131) in the 60 mg/day group. This increase was also evident at 9 months with a ratio (9 month:baseline) of 7.59 (95% CI 3.92 to 11.26, p=0.0038) in the 15 mg/day group to a mean ratio of 11.92 (95% CI 2.96 to 20.88, p=0.0228) in the 60 mg/day group. Linear regression shows a statistically significant association between higher dose and a higher 9-month:baseline ratio of β-carotene levels. In terms of vitamin E, there was a statistically significant increase in 3 month ratio to baseline in the 15 mg/day, 30 mg/day and 45 mg/day groups. Conversely, we found a statistically significant decrease in the 9 month ratio to baseline in all dose groups, including placebo. Linear regression shows a statistically significant association between higher dose and a lower 9-month:baseline ratio of vitamin E levels.

Conclusion: Supplementation with β-carotene at doses of 15, 30, 45, or 60 mg/day is effective at dramatically raising serum β-carotene at 3 and 9 months, and there is evidence of a dose-response relationship. Vitamin E levels declined in all groups at 9 months, following a transient increase at 3 month, with evidence of a small but significant association with β-carotene dose.

Comment [A1]: I would have stated this as "decreased risk of cancer associated with higher beta carotene intake", because we are going to intervene to increase intake rather than to decrease beta carotene levels. But your statements are corrct

Comment [A2]: It was actually plasma, but my documentation was inconsistent in that regard.

Comment [A3]: NO. Your primary comparison should be across dose groups, not time. That is, at a single point in time post randomization, we compare the dose groups to placebo. The analysis you present here does not tell us whether even the placebo group's means would have changed.

Comment [A4]: If I understand what you are doing here, this might be addressing the question. I note that I would have said something like "we measured the effect of treatment on each individual as the proportionate change in plasma beta carotene levels, We then compared that proportionate change across dose groups. You could give the mean ratios for representative dose groups (which MUST include placebo for this to have any scientific value.)

I note that as a general rule, analyzing ratios is less stable than analyzing differences, but in this data set (where measurements are far from 0), this is probably not too much of an issue.

Comment [A5]: So this highlights why we need to make the primary comparison across dose groups.

Comment [A6]: We need estimates and CI here. Is this at a level that we need to worry about toxicity?

Title

Effect of β-carotene supplementation on serum β-carotene and vitamin E levels in healthy adults.

Background

Cancer continues to produce a huge burden of morbidity and mortality within the United States and around the world. While there continue to be tremendous efforts directed at improving treatment for malignant disease, over the past several decades there has been increasing investigation into the prevention of cancer. There is also growing recognition that the public health impact of successful preventative strategies has the potential to dwarf the impact of improving cancer therapies. Ever since the link between smoking tobacco and the development of lung cancer was established through epidemiological studies in 1950, there has been more interest in the role that lifestyle plays in the causation of cancer. This focus on lifestyle has included investigations into the role that diet may play in the pathogenesis of cancer.

Numerous studies have investigated the role that vitamins play in modifying the risk of disease, particularly cancer, in humans. Recently, much of this attention has focused on vitamin A and its precursor, β-carotene, consumed in the human diet. Numerous *in vitro* experiments have shown promising characteristics of carotenoids, including enhancement of the immune response, inhibition of mutagenesis, and reduction of induced nuclear damage. Some carotenoids, especially β-carotene, powerfully reduce highly reactive singlet oxygen under certain conditions and can block free radical-mediated reactions that may play a central role in the pathogenesis of processes as diverse as malignant transformation and atherosclerosis.[1] Tissue concentrations of β-carotene have been found to be lower in cancers of the cervix, endometrium, ovary, breast, colon, lung, liver, and rectum compared to adjacent normal tissue.[2] Results from epidemiological studies over the past 15 years have intensified the interest in vitamin A and β-carotene. While there has been some evidence of an association between vitamin A and β-carotene and the risk of skin, colon, prostate, bladder and other cancers, the epidemiological evidence is strongest for an association in lung cancer.[2, 3] Several cohort studies have shown that lung cancer incidence varies inversely with β-carotene consumption as well as serum vitamin A levels.[4-9] A retrospective case-control study also found that patients with lung cancer had lower β-carotene and vitamin E levels than controls.[10] The inference of causation from these observational studies is relatively weak and needs to be bolstered by evidence from randomized, controlled trials of

β-carotene supplementation. However, more needs to be known about the pharmacokinetics of supplemental β-carotene in healthy humans before such a trial can be designed.

With any supplementation trial, consideration must be given to the potential for adverse effects. Although supplementation with β-carotene has been shown to be generally safe in animal and humans with no development of hypervitaminosis A[11], there has been some concern about the possibility that raising the concentration of β-carotene in the serum to supra-physiologic levels might lower the level of vitamin E, another fat soluble vitamin. In one study, supplementation with β-carotene in rats was associated with a 50% lower mean plasma vitamin E level than in rats who did not receive supplementation.[12] However, another study did not show a statistically significant decrease in vitamin E levels in rats supplemented with β-carotene.[13] It was noted though that this study's level of supplementation was 20 times less than the aforementioned study and lasted for just over half the amount of time. In a similar vein, rats supplemented with vitamin E had a 50% lower mean plasma β-carotene level than rats who did not receive supplementation.[14] Lastly, in a human study, supplementation with 30 mg/day of β-carotene showed a trend towards decreased vitamin E levels which did not achieve statistical significance, whereas in subjects receiving vitamin E supplementation carotenoid levels were found to be reduced by 20%.[15]

This interrelationship between Vitamin E and β-carotene is possibly related to the fact that both are polar molecules with similar modes of dietary uptake, and in serum both associate primarily with the low density lipoprotein (LDL) fraction of serum cholesterol.[16] In tissue both partition primarily to the plasma membrane, are found in the highest concentration in adipose tissue and the liver, and are present to a lesser extent in skeletal muscle and reproductive organs. It is unclear whether common cofactors are required for normal uptake, transport, sequestration and release of these molecules. Even if no such common factor is needed for β-carotene and vitamin E compartmentalization, serum levels might be antagonistic due to their parallel association with LDL. Excessive β-carotene supplementation might therefore conceivably lead to competitive inhibition of Vitamin E bioavailability, perhaps even to the point of deficiency among individuals with low serum cholesterol. Deficiency of vitamin E can have consequences ranging from subtle neurologic symptoms to ataxia and hemolytic anemia. Additionally, lower vitamin E levels have been associated with an increased risk of cancer.[10, 17] Thus, in addition to studying the pharmacokinetics of β-carotene, the impact of β-carotene supplementation on vitamin E serum levels should also be evaluated before cancer-related trials are considered.

Questions of Interest

The primary question of interest is how different doses of oral β-carotene in healthy adults affect serum β-carotene levels 3 and 9 months after the initiation of supplementation. We were also interested in whether β-carotene supplementation had any effect on plasma vitamin E levels.

Methods

Source of the Data

This data is drawn from a study of 46 healthy adult volunteers. At the initial study visit, patients had measurement of weight, body mass index, percent body fat, serum cholesterol level, and serum β-carotene and vitamin E levels. Each subject was then randomized to one of five groups defined by the dose of β-carotene to be taken: 0, 15, 30, 45, or 60 mg/day. The investigators and the patients were blinded as to the dose assignments, and the capsules for the 5 different dose levels were identical. The subjects each had blood samples drawn 3 months after initiating the treatment and again at 9 months after initiating the treatment. There was some drop out in the study, with 45 of the 46 subjects presenting for the 3-month blood-draw, and 40 of 46 presenting for the 9-month blood draw.

Statistical Methods

We first evaluated baseline characteristics of our five treatment groups in order to assess adequacy of our randomization, to evaluate whether baseline estimates and variability were comparable across strata, and whether any implausible or erroneous values were likely present. Arithmetic means, standard deviations and ranges were calculated for all continuous variables, while the means of binary variables are given as percentages. Baseline characteristics included sex, age, weight, body mass index (BMI), percent body fat, cholesterol, β-carotene level and vitamin E level.

Next, we determined the arithmetic mean, standard deviation and ranges for β-carotene and vitamin E levels associated with each treatment group at 3 and 9 months by treatment group.

Comment [A7]: OK to include when writing to a collaborator. This would be presumed (and not mentioned) when writing for a journal, unless you were using a data source known to be prone to error (and it was not your fault that it was)

Comment [A8]: We don't want a travelogue. We do want to know what you did.

We then sought to assess whether the changes in β-carotene and vitamin E levels at 3 and 9 months were significantly different from baseline values in each treatment group. Our primary analysis was evaluating the 3-months:baseline and 9-months:baseline ratios of β-carotene for all dose groups; we performed one-sample t-tests (allowing for unequal variances) to compare the observed ratios to 1 (the null hypothesis of no change over the study period). We also performed an analysis of 3-month:baseline and 9-month:baseline vitamin E levels for placebo and for all dose groups; again, the null hypothesis of no change from baseline was assessed using one-sample t-tests.

Since our primary interest was the long-term effect of β-carotene supplementation on serum beta-carotene levels and vitamin E, our further analyses were restricted to the 9-month:baseline ratio. We performed simple linear regression using robust standard errors to test for an association between dose and the ratio of 9-month to baseline β-carotene levels. We then used simple linear regression using robust standard errors to test for an association between dose and the ratio of 9-month to baseline serum vitamin E levels.

We also performed rudimentary exploratory analyses evaluating for potential effect modification of sex, baseline cholesterol, and percent body fat. The primary mechanism employed was to examine scatterplots of β-carotene and vitamin E 9-month:baseline ratios stratified by sex, baseline cholesterol, and percent body fat respectively. The latter two variables were dichotomized using *a priori* cutoffs of 220 mg/dL of cholesterol (using national guideline definition of elevated total cholesterol) and an arbitrary cutoff of 30% body fat.

All analyses were performed using Stata Software versions 9 and 10 (StataCorp, College Station, TX USA).

Results

Of the 46 patients who were enrolled, 45 were available for the 3-month blood sample with one of the patients randomized to the 60 mg/day group dropping out. Of the remaining 45 patients, 40 were available for the 9-month blood sample with one dropout in the placebo group, two in the 15 mg/day group, one in the 30 mg/day group, one in the 45 mg/day group, and no additional dropouts in the 60 mg/day group. Thus, there did not appear to be a trend in attrition with one to two dropouts in each group without a relationship to increasing dose.

Table 1 provides baseline characteristics of the study participants. However, given the patient who dropped out in 60 mg/day dose group provided no further data at 3 months and 9 months we removed her from our analysis of baseline characteristics. We were not concerned about any discrepancies in baseline characteristics; randomization appears to have been successful. While weight differed somewhat between the groups, the more accurate measures of adiposity, including BMI and percent body fat, were quite similar between groups. Differences in baseline β-carotene and vitamin E levels were not clinically significant. It is worth noting that there were differences between the treatment groups with respect to the proportion of women enrolled. However, from Figure 1 we do not see any obvious differences in the change in β-carotene or vitamin E levels when stratified by sex.

β-carotene

As can be seen from table 2, the mean β-carotene levels for the groups on supplementation had a dramatic rise in β-carotene levels by 3 months, which was sustained at the 9-month level (with continued supplementation). All 37 of the patients randomized to supplementation who had blood sampled at 3 months had a rise in serum β-carotene level from baseline, as did all 33 of those patients on supplementation who had blood sampled at 9 months; this contrasts starkly with the placebo group, six of whom had β-carotene levels below baseline at 3 and 9 months.

As per Table 3, evaluating the change in β-carotene level from baseline at 3 months via its ratio, we found a highly statistically significant increase in all groups receiving β-carotene supplementation and no significant change in the placebo group. The estimated mean 3-month ratios for the treatment groups ranged from 6.45 (95% CI 4.02 to 8.88, p= 0.0007) in the 15 mg/day group to 9.10 (95% CI 3.21 to 14.99, p=0.0131) in the 60 mg/day group. At nine months we again found highly statistically significant increases from baseline in all groups receiving supplementation. However, at nine months we found that the placebo group had a statistically significant decrease in β-carotene level compared to baseline, which was unexpected. The estimated mean 9-month ratios for the treatment groups ranged from 7.59 (95% 3.92 to 11.26, p= 0.0038) in the 15 mg/day group to 11.92 (95% CI 2.96 to 20.88, p=0.0228) in the 60 mg/day group.

Comment [A9]: Why ratios? This is a bit unusual, perhaps because ratios are often numerically unstable. You really ought to justify why you wanted the ratio.

Comment [A10]: Personally, such an analysis is possibly of interest descriptively, but it is not addressing our major scientific question, which instead is answered in an analysis comparing across dose groups (including placebo).

Comment [A11]: I agree that 9 months is of most interest. But additional questions would relate to: How early was an effect observed? Were the levels continuing to rise at the end of the study, i.e., do we need to worry about toxicity over a longer period?

Comment [A12]: very good to note

Comment [A13]: Do you think you gained or lost precision by looking at these ratios? How would you investigate this question?

Comment [A14]: This is why we have placebo groups. And this is what makes your omission of the placebo group from your abstract quite egregious.

(Diet varies over seasons, and beta carotene is something that people binge on—foods that have it, have a lot.)

Comment [A15]: You never tell us whether particular individuals had strikingly high or low values.

Using linear regression using robust standard errors, we did find evidence of a statistically significant association between β-carotene dose and the 9-month to baseline ratio of β-carotene levels. The estimated slope in our model was 0.1535; that is, for every 1 mg increase in dose we would expect our ratio of 9-month to baseline β-carotene levels to increase 0.1535. Our 95% confidence interval of 0.0369 to 0.2701 tells us that our observed slope of 0.1535 would not be unexpected if the true ratio increased by as little as 0.0369 per 1 mg increase in dose or as much as 0.2701 per 1 mg increase in dose. The p-value of 0.011 tells us we can confidently reject the null hypothesis of no association between the 9-month: baseline ratio of dose of supplemental β-carotene and the 9-month:baseline ratio of β-carotene levels.

Vitamin E

As can be seen from Table 2, it was common to see an initial increase in vitamin E levels at 3 months and a subsequent decline by 9 months, both in those on β-carotene supplementation and the placebo group, although to a lesser degree in the placebo group. The 3-month ratio for the treatment groups ranged from 1.14 (95% CI 1.04 to 1.24, p=0.0114) for the 15 mg/day group to 1.12 (95% CI 0.99 to 1.24, p=0.0691) for the 60 mg/day group. In evaluating the ratio of 3month:baseline vitamin E levels in each dose group we found a statistically significant increase in dose groups 15 mg/day, 30 mg/day and 45 mg/day from baseline, as can be seen in Table 3. When looking at the 9month to baseline ratio, we found a statistically significant decrease in the ratio for all dose groups as well as placebo. The 9-month mean ratios for the treatment groups ranged from 0.78 (95% CI 0.71 to 0.85, p=0.0002) in the 15 mg/day group to 0.77 (95% CI 0.68 to 0.86, p=0.0003).

Comment [A16]: what about the placebo group?

Using linear regression using robust standard errors, we did find evidence of a statistically significant association between β-carotene dose and the 9-month to baseline ratio of vitamin E. The estimated slope in our model was -0.0019; that is, for every 1 mg increase in dose we would expect our ratio of 9-month to baseline β-carotene levels to decrease by 0.0019. Our 95% confidence interval of -0.0034 to -0.0003 tells us that our observed slope of -0.0019 would not be unexpected if the true ratio decreased by as little as 0.0034 per 1 mg increase in dose or as much as 0.0003 per 1 mg increase in dose. The p-value of 0.018 tells us we can confidently reject the null hypothesis of no association between the 9-month: baseline ratio of dose of supplemental β-carotene and the 9-month:baseline ratio of β-carotene levels.

Comment [A17]: beta carotene or vitamin E?

We did also perform exploratory analyses for effect modification, the results of which can be seen in Figure 1. We were specifically interested in seeing if the effect of increasing dose on the 9-month to baseline ratios (increasing the ratio in β-carotene and decreasing it in vitamin E) was modified by sex, baseline cholesterol level, or percent body fat. Although interpretation of scatterplots with smoothers should always be done with extreme caution (and even moreso given our small sample sizes), we can comfortably report the absence of evidence of any such interaction based upon our analyses.

Discussion

We presented evidence that β-carotene supplementation, at doses of 15, 30, 45, and 60 mg/day, is effective at dramatically raising β-carotene levels. This effect appears within three months of starting supplementation and continues at 9 months (with continued β-carotene supplementation). The statistically significant decline in serum β-carotene levels seen in the placebo group in this study at nine months was an unexpected result, and likely reflects the effect of a changing environmental or dietary factor unrelated to the execution of this study. There does appear to be a dose-relationship, since our regression model showed a positive association between dose and increasing 9-month:baseline ratio. The goal of β-carotene supplementation is to achieve a lowering of cancer risk, which would confirm the inference of causation of the association between low β-carotene levels and higher cancer rates. It is important to keep in mind that none of the epidemiological studies have shown a difference in serum β-carotene levels between low and high risk individuals that even approaches what we see between baseline and 9 months in the 15 mg/day group in our study (a 7.6-fold increase, 95% CI 3.9 to 11.3, p=0.004). Thus, it is not clear to us that the 11.9-fold increase (95% 2.96 to 20.88) seen in the 60 mg/day group is preferable. Since we are not certain that the statistically significant degree of dose response is clinically significant, and since even the lowest dose had a dramatic effect on serum β-carotene levels, we advocate following the pharmacological principle of using the lowest dose shown to be effective, to minimize the risk of adverse effects (both known and unknown). If a more precise evaluation of the differential effects of different doses is truly needed, then an additional trial with more participants is needed.

Comment [A18]: continues or increases?

Comment [A19]: Is our ratio high because we started with beta carotene deprived individuals? That is an easy way to get a high ratio.

Because vitamin E has also been implicated as having a possible role in cancer prevention, and since some evidence exists about the potential of supplemental β-carotene to lower serum vitamin E levels, it was necessary that we address the potential for this adverse effect. Significant increases were seen in vitamin E levels between baseline and 3 months in the 15 mg/day, 30 mg/day and 45 mg/day dose groups. It is possible that the increase seen in the 60 mg/day group did not reach statistical significance because of our limited number of subjects. However, it could also be secondary to this higher dose of β-carotene supplementation competing with vitamin E absorption or transport. Seeing such an increase in any group was an unanticipated outcome, and we are not certain of the cause. At the 9 month interval we detected a statistically significant decrease in vitamin E levels in all dose groups from baseline, *including the placebo group*. As with β-carotene levels, the fact that the placebo group had a significant decline in vitamin E levels while on study may implicate a changing environmental or dietary factor not necessarily related to participation in this study. Our linear regression model does demonstrate a dose response, in that higher doses saw a statistically significantly lower 9-month:baseline ratio of vitamin E levels. It is important to consider the degree of decline that was seen. The decline was from a maximum 26% average decline from baseline in the 45 mg/day dose group (95% CI 17-35%) compared to an average 10% decline in the placebo group (95% CI 3-17%), and the linear regression model shows a very small dose-response association. While we can safely say that the decline seen here would not be expected to cause other adverse effects seen with true vitamin E deficiency, we are concerned that the magnitude of the decline in vitamin E levels (approaching the mean difference from epidemiological studies of breast cancer patients versus controls[17]) does raise the concerning possibility that the net effect of β-carotene supplementation on cancer risk. The effect of β-carotene supplementation on serum vitamin E levels warrants further study.

Comment [A20]: Is toxicity associated with a proportionate decline, or with hitting some absolute level? In either case, quantify what that would be. Also we need to discuss whether individual patients had too much of a decline.

Conclusion

We found that β-carotene at daily doses of 15, 30, 45, or 60 mg dramatically raises serum β-carotene levels at 3 and 9 months, and that higher doses are associated with higher 9-month to baseline serum β-carotene ratios. We also found that while vitamin E tends to increase at 3 months, by 9 months all groups (including placebo) had experienced a significant decline in vitamin E levels. Higher doses were shown to be associated with a very small but statistically significantly lower 9-month:baseline serum vitamin E ratio.

References

1 Bendich A, Olson JA. "Biological actions of carotenoids." *FASEB J.* 1989 Jun; 3(8):1927-32.

2 Peto R, *et al.* "Can dietary beta-carotene materially reduce cancer rates?" *Nature.* 1981 Mar 19; 290(5803):201-8.

3 Kummet T, *et al. "*Vitamin A: evidence for its preventive role in human cancer". *Nutr Cancer*. 1983; 5(2):96-106.

4 Palan PR, *et al*. "Decreased beta-carotene tissue levels in uterine leiomyomas and cancers of reproductive and nonreproductive organs*".* *Am J Obstet Gynecol.* 1989 Dec; 161(6 Pt 1):1649-52.

5 Bjelke E. "Dietary vitamin A and human lung cancer". *Int J Cancer.* 1975; 15:561-65.

6 Kvale G, *et al.* "Dietary habits and lung cancer risk." *Int J Cancer.* 1983; 31:397-405.

7 Mettlin C, *et al* "Vitamin A and lung cancer." *JNCI.* 1979; 62:1435-38.

8 Gregor A, *et al*. "Comparison of dietary histories in lung cancer cases and controls with special reference to vitamin A." *Nutr Cancer.* 1980; 2:93-97

9 Connett JE, *et al*. "Relationship between carotenoids and cancer. The Multiple Risk Factor Intervention Trial (MRFIT) Study." *Cancer*. 1989 Jul 1; 64(1):126-34.

10 Menkes M, et al. "Serum β-carotene, vitamins A and E, selenium, and the risk of lung cancer." *NEJM.* 1986; 315:1250-4.

11 Bendich A. "The safety of β-carotene." *Nutrition and Cancer*. 1988; 11(4):207-14.

12 Bendich A, Shapiro S. "Effect of β-carotene and canthaxanthin on the immune responses of the rat." *J Nutr.* 1986; 116:2254-62.

13 Alam S, et al. "Lipid peroxide, α-tocopherol and retinoid levels in plasma and liver of rats fed diets containing β-carotene and 13-cis-retinoic acid." *J Nutr.* 1983; 113:2608-14.

14 Alam B, et al. "Influence of dietary fats and vitamin E on plasma and hepatic vitamin A and β-carotene levels in rats fed excess β-carotene." *Nutrition and Cancer.* 1990; 14:111-6.

15 Willett W, et al. "Vitamins A, E, and carotene: effects of supplementation on their plasma levels." Am J Clin Nutr. 1983; 38:559-66.

16 Bjørneboe A, Bjørneboe GA, Drevon CA. "Absorption, Transport and Distribution of Vitamin E." *J Nutr.* 1990 Mar; 120(3):233-42.

17 Wald N, et al. "Plasma retinol, β-carotene and vitamin E levels in relation to the future risk of breast cancer." Br J Cancer. 1984; 49:321-4.

Table 1. Baseline characteristics by dose group

	Placebo (n=8)	15mg/day (n=10)	30mg/day (n=10)	45mg/day (n=8)	60mg/day (n=9)
Mean +/- SD (min, max)					
Age (yrs)	56.3 ± 4.3 (52, 64)	56.3 ± 4.6 (50, 62)	57.2 ± 4.1 (50,64)	55.9 ± 3.1 (51, 60)	55.6 ± 4.5 (52, 64)
% Male	62.5%	50%	30%	50%	44%
Weight	180 ± 33 (118, 229)	167.8 ± 36.8 (118, 213)	151.8 ± 30.2 (123, 204)	172.6 ± 40.9 (126, 253)	156 ± 16.7 (126, 177)
BMI	26.6 ± 3.6 (19.7, 30.4)	25.7 ± 3.6 (20.7, 31.7)	25.6 ± 2.6 (22.4, 31.5)	25.3 ± 3.3 (21.7, 30.9)	24.5 ± 2.1 (21.7, 28.7)
Cholesterol	217.8 ± 28.5 (190, 283)	223 ± 29.7 (171, 265)	213.2 ± 33.5 (159, 268)	213.3 ± 33.5 (169, 263)	231 ± 33 (209, 313)
% body fat	0.28 ± 0.08 (0.17, 0.42)	0.28 ± 0.09* (0.16, 0.44)	0.30 ± 0.06 (0.22, 0.37)	0.32 ± 0.06 (0.27, 0.43)	0.30 ± 0.1 (0.18, 0.43)
β-carotene	270 ± 136 (136, 476)	220 ± 128 (65, 496)	219 ± 84 (126, 349)	227 ± 106 (93, 396)	235 ± 115 (48, 408)
Vitamin E	7.88 ± 1.42 (6.19, 10.71)	7.76 ± 1.21 (5.10, 9.24)	7.98 ± 1.62 (5.12, 9.46)	8.24 ± 0.95 (7.22, 10.05)	8.3 ± 1.27 (6.32, 10.7)

* Missing value for one subject.

Table 2. Serum β-carotene and vitamin E levels by dose group at baseline, 3 months and 9 months

Dose	Baseline	3 months	9 months
β-carotene levels (mg/dL), Mean ± SD (min, max)			
Placebo	270 ± 136 (136, 476)	244 ± 94 (109, 384)	186 ± 88 (85, 323)
15 mg/day	220 ± 128 (65, 496)	1116 ± 317 (699, 1603)	1254 ± 571 (577, 2019)
30 mg/day	219 ± 84 (126, 349)	1302 ± 260 (854, 1603)	1505 ± 479 (849, 2249)
45 mg/day	227 ± 106 (93, 396)	1236 ± 239 (861, 1442)	1749 ± 579 (950, 2310)
60 mg/day	218 ± 122 (48, 408)	1467 ± 251 (1098, 1960)	1878 ± 430 (1233, 2855)
Vitamin E levels (mg/L), Mean ± SD (min, max)			
Placebo	7.88 ± 1.42 (6.19, 10.71)	8.27 ± 1.23 (6.50, 10.11)	7.25 ± 1.13 (5.26, 8.93)
15 mg/day	7.76 ± 1.21 (5.10, 9.24)	8.71 ± 0.91 (6.36, 9.74)	5.75 ± 0.50 (4.61, 6.28)
30 mg/day	7.98 ± 1.62 (5.12, 9.46)	9.15 ± 0.90 (7.12, 10.55)	6.30 ± 1.14 (4.31, 7.74)
45 mg/day	8.24 ± 0.95 (7.22, 10.05)	8.98 ± 0.63 (7.89, 9.78)	6.15 ± 0.88 (4.94, 7.05)
60 mg/day	8.44 ± 1.27 (6.32, 10.71)	9.11 ± 0.66 (8.07, 10.02)	6.32 ± 1.12 (4.87, 8.06)

Comment [A21]: would this be toxically high?

Comment [A22]: would this be toxically low?

Table 3. Ratio of β-carotene and vitamin E levels from baseline to 3 and 9 months, with one-sample tests of hypotheses of no change from baseline

Dose (mg/day)	**Ratio 3 month:baseline**[†]		**Ratio 9 month:baseline**[†]	
β-carotene				
	Point estimate, (standard error)	*95% Confidence Interval, (two sided p-value)*	*Point estimate, (standard error)*	*95% Confidence Interval, (two sided p-value)*
Placebo	0.96 (0.09)	0.75 to 1.17, (0.6914)	0.67 (0.07)	0.49 to 0.86, (**0.0047**)
15	6.45 (1.07)	4.02 to 8.88, (**0.0007**)	7.59 (1.55)	3.92 to 11.26, (**0.0038**)
30	6.45 (0.59)	5.12 to 7.78, (**<0.0001**)	7.20 (0.76)	5.45 to 8.95, (**<0.0001**)
45	6.21 (0.68)	4.60 to 7.81, (**0.0001**)	8.45 (0.81)	6.46 to 10.44, (**0.0001**)
60	9.10 (2.55)	3.21 to 14.99, (**0.0131**)	11.92 (3.89)	2.96 to 20.88, (**0.0228**)
Vitamin E				
	Point estimate, (standard error)	*95% Confidence Interval, (two sided p-value)*	*Point estimate, (standard error)*	*95% Confidence Interval, (two sided p-value)*
Placebo	1.06 (0.03)	0.98 to 1.13, (0.109)	0.90 (0.03)	0.83 to 0.97, (**0.0108**)
15	1.14 (0.04)	1.04 to 1.24, (**0.0114**)	0.78 (0.03)	0.71 to 0.85, (**0.0002**)
30	1.18 (0.05)	1.05 to 1.30, (**0.0102**)	0.83 (0.06)	0.69 to 0.96, (**0.0197**)
45	1.10 (0.03)	1.02 to 1.17, (**0.0188**)	0.74 (0.04)	0.65 to 0.83, (**0.0003**)
60	1.12 (0.06)	0.99 to 1.24, (0.0691)	0.77 (0.04)	0.68 to 0.86, (**0.0003**)

[†] **One-sample T-test with null hypothesis that ratio equals one**

Figure 1: Scatterplots of 9-month beta-carotene (left) and vitamin E (right) versus dose, stratified by sex (row 1), baseline serum cholesterol [</= or > 220 mg/dL] (row 2), and baseline percent body fat [</= or > 30%] (row 3)