

Effects of multivitamin/mineral supplementation on plasma levels of nutrients. Report No. 4 of the Italian-American Clinical Trial of Nutritional Supplements and Age-related Cataract

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Summary. The use of multivitamin-mineral supplements has become increasingly common, but whether the use of such supplements improves micronutrient status remains still unclear. The objective of this report is to investigate how a long-term vitamin-mineral supplementation following the US Recommended Daily Intake (RDI) affected the plasma levels of selected nutrients in a subset (No. = 407) of participants in the Italian-American Clinical Trial of Nutritional Supplements and Age-related Cataract (CTNS). The CTNS was a double-blind, single centre, controlled clinical trial of 1020 participants aged 55-75 years randomized to a daily tablet of Centrum® or placebo. A representative sample of 40% of the 1020 subjects, whom plasma level of selected vitamins was determined at the baseline, was retested throughout the treatment period that averaged 9.0 ± 2.4 years. Participants assigned to Centrum® showed a significant increase ($p < 0.005$) in mean/median plasma levels of vitamin E, beta-carotene, folate, and vitamin B12, and an improved riboflavin status when compared with participants assigned to placebo. Differences concerning vitamin C were statistically less relevant and those concerning vitamin A were at a borderline level. In the treated group the effect of supplementation on plasma levels of vitamins A, E, and C, and on the glutathione reductase activation coefficient was significantly higher in participants with lower nutritional status at baseline.

Key words: clinical trial, dietary supplementation, epidemiology, vitamin level.

Riassunto (*Effetto dell'integrazione della dieta con multivitaminici e minerali sui livelli di nutrienti nel plasma. Rapporto No. 4 dell'Italian-American Clinical Trial of Nutritional Supplements and Age-related Cataract*). Sebbene sia sempre più diffuso l'uso di integratori multivitaminici e minerali nella dieta, ancora non è stato chiarito se tali integratori favoriscano lo stato dei micronutrienti plasmatici. Questo rapporto si propone di descrivere come l'integrazione continuativa con vitamine e minerali secondo i livelli di assunzione giornaliera raccomandati negli USA (US RDI) abbia modificato, in un gruppo (No. = 407) di soggetti partecipanti all'*Italian-American Clinical Trial of Nutritional Supplements and Age-related Cataract* (CTNS), le concentrazioni plasmatiche dei nutrienti analizzati. Il CTNS è consistito in una sperimentazione clinica controllata e randomizzata monocentrica in doppio cieco. I soggetti partecipanti, di età tra 55-75 anni, per metà sono stati trattati durante un periodo di $9,0 + 2,4$ (media + DS) anni con una compressa al giorno dell'integratore dietetico "Centrum" mentre un placebo era assegnato ai restanti soggetti. Un campione rappresentativo pari al 40 per cento dei 1020 soggetti per i quali era stato determinato al baseline il livello nel plasma di una selezione di vitamine è stato ripetutamente riesaminato durante il periodo di trattamento. I partecipanti assegnati al Centrum hanno avuto un incremento significativo ($p < 0,005$) nella media o mediana dei livelli plasmatici di vitamina E, beta-carotene, folati, e vitamina B12, ed una significativa riduzione del coefficiente di attivazione della glutazione riduttasi (indice di una maggiore disponibilità di riboflavina) rispetto ai partecipanti assegnati al placebo. Differenze statisticamente meno significative hanno riguardato la vitamina C ed erano a livelli *borderline* per la vitamina A. Nel gruppo dei trattati l'effetto dell'integrazione dietetica sui livelli nel plasma delle vitamine A, E, e C, e sul coefficiente di attivazione della glutazione riduttasi è stato significativamente più elevato in quelli con un più basso livello nutrizionale all'inizio dello studio.

Parole chiave: sperimentazione clinica, integratori dietetici, epidemiologia, vitamine.

INTRODUCTION

The use of dietary supplements has become increasingly common in the United States and Europe, especially among older persons. In the 1999-2000 National Health and Nutrition Examination Survey, a nationally representative, cross-sectional survey of US health and nutrition, 52% of adults reported taking a dietary supplement in the past month [1]. Thirty-five per cent took a multivitamin-multimineral supplement. Most supplements were taken daily and for at least 2 years. In general, supplement use has been more common in women, older age groups and persons with higher education levels, higher dietary nutrient intakes and healthier diets [2]. In a 2002 health and diet survey conducted by the US Food and Drug Administration, 73% of US non-institutionalized adults aged 18 years or older had used a dietary supplement in the previous 12 months [3]. Eighty-five percent of supplement users reported taking a multivitamin/multimineral supplement. Supplement use has been reported to be somewhat lower in Europe with 35% of the overall population ≥ 18 years in England in 2004 regularly taking at least one dietary supplement, and with 51% of women and 34% of men among free-living 60+ year-old persons in Germany in 2006 taking at least one supplement during a three days estimated dietary record [4, 5]. The use of dietary supplements is becoming more common also in selected Asian populations as reported in the Elderly Nutrition and Health Survey in Taiwan (1999-2000) [6]. Suggestions of beneficial effects of various nutrients on age-related diseases such as cataract, cardiovascular disease and cancer have contributed to the growing number of elderly who use dietary supplements [7-9]. In spite of the growing popularity of supplement use limited data are available on how the most popular formulations, which contain only RDA amounts of nutrients, affect plasma levels in well nourished, healthy individuals and how lifestyles such as smoking and alcohol use affect those blood levels.

The Italian-American Clinical Trial of Nutritional Supplements and Age-Related Cataract (CTNS) was designed to test whether use of Centrum® (Wyeth Consumer Healthcare, Madison, NJ), a popular multivitamin/mineral supplement containing US Recommended Daily Intake (RDI) levels of nutrients, could influence the incidence and progression of age-related lens opacities. Results from the trial have been previously published [10]. We are reporting here the effect of Centrum on the blood levels of several micronutrients in participants randomly assigned to placebo or Centrum tablets over a follow-up period that averaged 9.0 ± 2.4 years.

PARTICIPANTS AND METHODS

Study population

Details of the trial design and methods were presented elsewhere [11] and are briefly summarized here. The Institute of Ophthalmology at the

University of Parma, Italy, enrolled 1,020 participants aged 55 to 75 years from January 8, 1996, through April 10, 1998. The last follow-up visit occurred on May 25, 2007. Persons with no cataract or early cataract according to study definitions were enrolled. Ocular exclusion criteria included presence of advanced cataract or eye conditions that could interfere with the prospective evaluation of lens changes. General exclusion criteria were current use of dietary supplements containing nutrients in the study medication, conditions or circumstances that might interfere with participant follow-up, and refusal to sign the informed consent. The study protocol was approved by an independent data and safety monitoring committee and by the Ethics Committee of the University of Parma. The trial was registered at the NIH Registry, (www.clinicaltrials.gov), in September 2005 (number NCT00309387).

Interventions/randomization

Participants were assigned to one daily tablet of Centrum®, a multivitamin/mineral supplement containing US RDI levels of nutrients (*Table 1*) or a matched placebo tablet. Treatment assignments were double masked.

Procedures and follow-up

Medical history and history of drug use, smoking habits, alcohol use, and sunlight exposure were collected at the randomization visit on all participants. Blood pressure, height and weight were also measured. Follow-up visits were performed at 6-month intervals. Information on dietary intake was collected by direct interview from a randomly selected subset of 346 participants at the third (18th month) or seventh (42nd month) follow-up visit by administering a 24 hour recall questionnaire (EPIC SOFT) specifically developed for use in European populations [12].

Blood samples for collecting biochemical nutrient data on plasma (vitamins A, E, C, and betacarotene) and red blood cells (glutathione reductase activation coefficient, GRAC) were drawn from all participants at baseline and from two subsets; subset 1 (No. = 204) at the first, third, sixth and eighth annual visit, and subset 2 (No. = 203) at the second, fourth, sixth and eighth annual visit. Thus, for subset 1 the first follow-up biochemical assessment was done at year 1, and for subset 2 at year 2. Folate and vitamin B12 levels were also assessed on subset 1 participants at baseline and on both subsets during follow-up (according to the above mentioned scheme) as potentially useful for monitoring compliance with study treatment regimen. Compliance with the treatment regimen was also assessed by tablet counts at each follow-up visit.

Biochemical analyses

Fasting blood samples were collected in EDTA tubes. After separation of plasma (2,500 rpm for 10 min) red blood cells were washed twice in phosphate-buffered saline and stored in a β -mercap-

Table 1 | Composition of multivitamin-mineral supplement (Centrum®)

Substances	Amount	DV (%)
Vitamin A	5,000 IU	100
Vitamin E	30 IU	100
Vitamin C	60 mg	100
Folic acid	400 mcg	100
Vitamin B ₁	1.5 mg	100
Vitamin B ₂	1.7 mg	100
Niacinamide	20 mg	100
Vitamin B ₆	2 mg	100
Vitamin B ₁₂	6 mcg	100
Vitamin D	400 IU	100
Biotin	30 mcg	10
Pantothenic acid	10 mg	100
Calcium	162 mg	16
Phosphorus	125 mg	13
Iodine	150 mcg	100
Iron	18 mg	100
Magnesium	100 mg	25
Copper	2 mg	100
Zinc	15 mg	100
Manganese	2.5 mg	125
Selenium	25 mcg	35
Chromium	25 mcg	21
Vitamin K	25 mcg	31
Molybdenum	25 mcg	33
Chloride	36.3 mg	1
Potassium	40 mg	1

DV: daily value. This value is based on the US RDI levels as established by the US Food and Drug Administration. The tablet also contains trace amounts of the following substances for which no US RDI doses have been established: nickel, tin, silicon, vanadium, and boron.

toethanol-EDTA stabilizing solution. All samples were stored at -80 °C. HPLC was used to analyze vitamin A (retinol), beta-carotene, and vitamin E (alpha-tocopherol) plasma levels after extraction with hexane, solvent evaporation under nitrogen, and reconstitution in 200 µl of the mobile phase. The analysis was performed using reverse-phase Waters Symmetry C-18 columns (3.9 × 5 µm particle size) and a pre-column Sentry Symmetry C-18 (3.9 × 20 mm) (Italy Waters SPA, 20090 Vimodrone, Italy) and isocratic elution with acetonitrile/methanol/ethanol (350:300:350). The chromatographic equipment included a double pump (Beckman System Gold 1255) (Beckman Instruments Inc, CA, USA), a Waters 717 autosampler and a 975 Intelligent UV-Visible Detector (Jasco Corporation, 2967-5, Ishikawa-cho, Hachioji, Tokyo, Japan) set a 325 nm (retinol), 290

nm (alpha-tocopherol) and 450 nm (beta-carotene) allowing performance of all analyses in the same run (flow rate 1 ml/min; total run time 13 min). Retention time was 2.1 min for vitamin A, 4.6 min for vitamin E, and 12 min for beta-carotene.

For vitamin C analysis one volume of plasma was added to four volumes of a 6% solution of metaphosphoric acid before storage at -80 °C. Before testing, after thawing and centrifugation (2,500 rpm), the supernatant (100 µl) was mixed with a solution of trisodium phosphate and dithiothreitol (300 µl) to reduce dehydroascorbate to ascorbate and then re-acidified with 40% metaphosphoric acid solution. The analysis was carried out with isocratic HPLC using an analytic reverse phase column Waters Resolve C-18 (3.9 × 150 mm, 5 µm particle size), a Resolve C-18 (3.9 × 20 mm) pre-column and a saline mobile phase (pH 3.0) containing 0.15 M monochloroacetic acid, 2 mM sodium EDTA and 0.13 mM octylsulfonic acid [13]. An electrochemical Waters 464 (Div. Millipore/Waters Chromatography, Milan, Italy) set at 650 mV was utilized. Flow rate was 1 ml/min, run time 10 min, and vitamin C retention time 1.7 min.

Prior to each analysis session a calibration curve for each nutrient was performed to calculate plasma concentration.

After disrupting red blood cells by two cycles of freeze and thaw in liquid nitrogen, erythrocyte glutathione reductase (GR) activity with and without added FAD was assessed by measuring reduced nicotinamide-adenine-dinucleotide phosphate oxidation at 340 nm in the presence of oxidized glutathione [14]. GR activity was expressed as units/g Hb and calculated as activity with FAD / activity without FAD (GR activation coefficient, GRAC). Higher levels of FAD-induced activation result therefore in higher test values and are considered suggestive of low-riboflavin status. Hb concentration was assessed by the cyanomethemoglobin method using Drabkin's reagent (kit 525-A, Sigma Diagnostic, Sigma Aldrich srl, 20151 Milan, Italy).

Quality control

Masked replicates (20 for each micronutrient) were performed before the start of the study and intra-class correlations were calculated (0.94 for vitamin A, 0.95 for vitamin E, 0.96 for beta-carotene, 0.97 for vitamin C and GR, and 0.91 for GR with added FAD). Ongoing assessment of reproducibility was performed by regularly including as masked replicates about 7% of study samples. "End-of-study" reproducibility analysis by intra-class correlation was 0.96 (No. = 189) for vitamin A and vitamin E; 0.95 (No. = 189) for beta-carotene; 0.97 (No. = 113) for vitamin C; 0.97 and 0.96 (No. = 186) for GR with and without FAD respectively; 0.98 (No. = 46) for vitamin B12; and 0.94 (No. = 46) for folate.

During the second study year a slight but progressive loss of resolution of the HPLC column used for vitamin C measurement was observed on the chromatographic curves. A new chromatographic

column was used to retest the samples (No. = 411) from this period and the new data were substituted for the previous ones after further analysis of a subset of study samples confirmed that sample storage did not influence the results. From then on utilization time of chromatographic column was limited to 150 analyses for vitamin C and 300 analyses for the other vitamins.

Statistical analysis

Box-plots were used for the descriptive analyses. The horizontal line inside the box indicates the median value. The inner box indicates the inter-quartile range that runs between the 25th to 75th percentiles. The upper line extending from the box indicates the largest value between the 75th percentile and the point that is 1.5 times the inter-quartile range. The lower line extending from the box indicates the smallest point between the 25th percentile and 1.5 times the inter-quartile range. The circles represent values that lie outside these ranges. Extreme outliers were excluded to improve the graphical representations and were not included in the statistical test calculations. Extreme outliers were defined as those exceeding 3 times the inter-quartile range.

In the subgroup analyses, we designated as the 1st, 2nd, 3rd, and 4th quartiles the sets of values defined by the median and the lower and upper quartiles. In order to define current, past and never drinkers or smokers the following definitions were used: “smoked at least one cigarette a day for at least one year”; “drank wine, beer, or spirits at least once a week, for at least one year”.

Statistical significance of differences was estimated with the Student’s t test when the data distribution was normal; otherwise with the Kruskal-Wallis test. Differences were considered statistically significant at $p < 0.05$. Analyses were performed using STATA Statistical Software.

RESULTS

A total of 1020 participants (mean age 68 ± 5 years, 55% males) were enrolled and followed for a mean follow-up time of 9.0 ± 2.4 years. By the end of follow-up 145 (14.2%) had died, 156 (15%) were nominally lost to follow up, and 174 (17%) stopped taking CTNS treatment, including 80 who were given the option to stop after cataract surgery on the study eye(s). By the end of the study 122 participants (12.0%) had taken non-study nutrient supplements during the trial (11.4% and 12.5% in treated and control groups, respectively).

Compliance with the treatment regimen by year of follow-up and treatment arm is summarized in *Figure 1*, which shows that more than 50% of participants took more than 90% of their study tablets (median 91%).

Dietary intake, as assessed with the 24 hour recall questionnaire in conjunction with USDA conversion tables, was in good agreement with that reported in

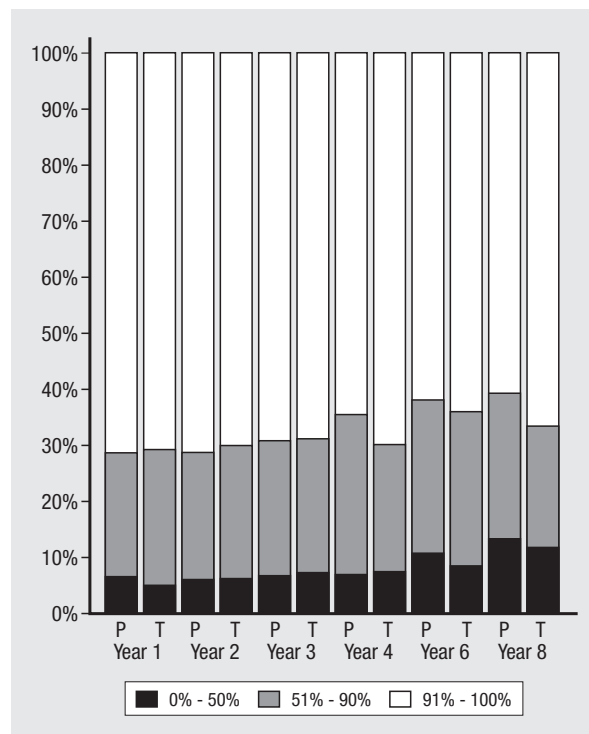


Fig. 1 | Compliance with treatment regimen by year of follow-up* and treatment assignment** in CTNS.

*Includes only follow-up years in which blood samples were collected. **P: placebo; T: Treatment.

comparable population samples of Northern Italy [15]. Average daily intake of nutrients for which biochemical data were calculated is reported in *Table 2*.

Plasma levels of nutrients were balanced across treatment arms at baseline. Females showed higher levels of vitamin C, alpha-tocopherol and beta-carotene ($p < 0.005$). Distribution of baseline plasma nutrient and GRAC values for the whole sample were compared with the currently accepted deficiency threshold values [16]. Two participants of 1016 (0.2%) had vitamin E levels $< 11.6 \mu\text{Mol/L}$, none had vitamin A levels $< 0.7 \mu\text{Mol/L}$, 111 of 1010 (11.0%) had vitamin C levels $< 17 \mu\text{Mol/L}$, 1 of 203 (0.5%) had vitamin B12 levels $< 74 \text{pMol/L}$, 71 of 206 (34.5%) had folate levels $< 6.8 \text{nMol/L}$, and 252 of 1017 (24.8%) had a GRAC value > 1.2 .

Mean baseline plasma levels of vitamins A, E, C, B12, beta-carotene, folate, and erythrocyte GRAC for the whole cohort and mean follow-up plasma levels of the same nutrients for subgroups 1 and 2 are presented in *Figures 2-8* and in *Supplemental Tables 1-7**. Mean change from baseline for the two subgroups during follow-up are shown in the tables. The tables exclude extreme outliers (vitamin A, N

* *Supplemental Tables 1-14 will be available in the online version of this article at www.iss.it/lanna*

Table 2 | Dietary intake of nutrients for which biochemical data were collected in the CTNS cohort (24 hour recall)

	No. of observations	Mean	5th percentile	95th percentile
Vitamin A IU/day*	346	5617.80	1002	15915
α -tocopherol mg/day	346	7.08	2.892	14005
Ascorbic acid mg/day	346	101.42	16.21	277.12
Riboflavin mg/day	346	1.61	0.79	2.71
Vitamin B12 μ g/day	346	5.25	0.88	15.31
Folate μ g/day	346	294.42	128	536

*IU corresponds to 0.3 μ g of retinol in foods of animal origin and to 0.6 μ g of beta-carotene in foods of vegetable origin.

= 1 (P); vitamin E, No. = 1 (P), No. = 5 (T); beta-carotene, No. = 7 (P), N = 21 (T); vitamin C, No. = 1 (T); GRAC, No. = 11 (P), No. = 7 (T); vitamin B12, No. = 3 (P), No. = 26 (T); folate, No. = 3 (P), No. = 11 (T)). Inclusion of extreme outliers did not appreciably affect the results.

In the placebo group, as expected, mean plasma values of the two subgroups with follow-up data did not show relevant changes compared with baseline values for any of the nutrients. Change from baseline during follow-up was significantly increased in those assigned to Centrum compared with those assigned to placebo ($p < 0.005$) for vitamin E, beta carotene, vitamin B12 and folate, and significantly decreased for GRAC (indicating improved riboflavin status). A greater change from baseline was also present in those assigned to Centrum compared with those assigned to placebo in four and in three of the six follow-up visits for vitamin C ($p < 0.05$ and $p < 0.005$) and for vitamin A ($p < 0.05$), respectively. Gender did not appreciably influence plasma level response to supplementation (follow-up years 1-2) for vitamin A, E, C, GRAC, and folate. Females showed higher in-

creases in plasma levels of beta-carotene and vitamin B12 following supplementation (data not shown).

Stratifying by smoking habits (smokers/ ex smokers/ never smoked) or alcohol use (drinkers/ ex drinkers/ never drinkers) did not appreciably influence the results (data not shown).

We tested whether baseline plasma levels influenced the effect of supplementation by comparing changes in plasma levels between baseline and follow-up years one and two in participants with baseline plasma values in the 1st quartile vs participants with baseline plasma levels in the 4th quartile (*Supplemental Tables 8-14*). Moderate changes during follow-up, consistent with regression towards the mean, were observed in both the 1st and 4th quartiles of the group assigned to placebo for all nutrients. For those assigned to Centrum increases in plasma levels were significantly greater in persons in the 1st compared with the 4th quartile for vitamin A, E, C, and GRAC, suggesting a greater effect of supplementation in participants with lower nutritional status at baseline.

The effect of supplementation was similar for participants in the 1st and 4th quartiles at baseline for

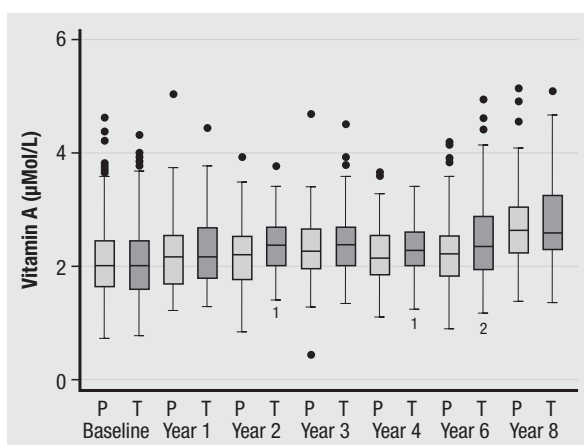


Fig. 2 | Baseline and follow-up mean levels of plasma vitamin A in placebo (P) and Centrum (T) treated subjects in CTNS. P vs T change from baseline; 1 = t test - $p < 0.05$; 2 = Kruskal-Wallis test - $p < 0.05$.

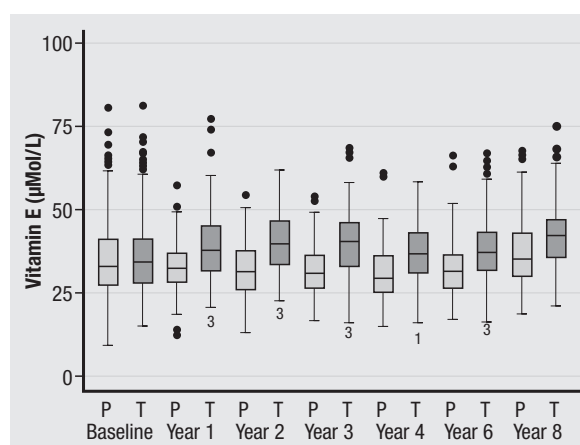


Fig. 3 | Baseline and follow-up mean levels of plasma vitamin E in placebo (P) and Centrum (T) treated subjects in CTNS. P vs T change from baseline; 1 = t test - $p < 0.05$; 3 = t test - $p < 0.005$.

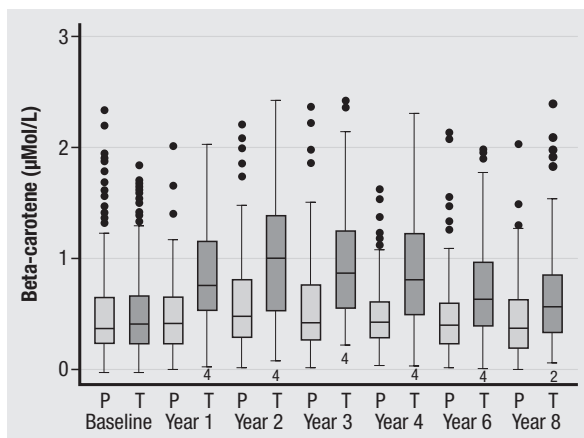


Fig. 4 | Baseline and follow-up mean levels of plasma beta-carotene in placebo (P) and Centrum (T) treated subjects in CTNS. P vs T change from baseline; 2 = Kruskal-Wallis test - $p < 0.05$; 4 = Kruskal-Wallis test - $p < 0.005$.

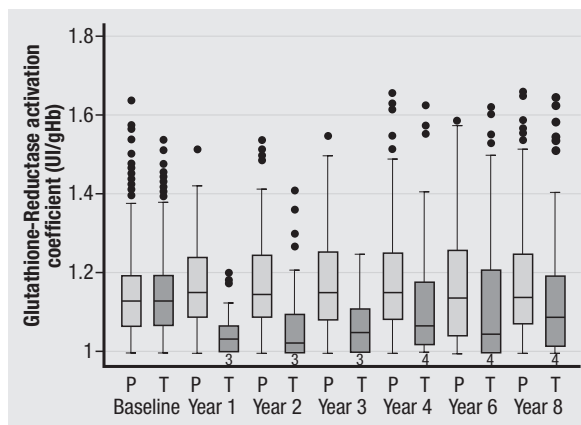


Fig. 6 | Baseline and follow-up mean levels of plasma glutathione-reductase activation coefficient in placebo (P) and Centrum (T) treated subjects in CTNS. P vs T change from baseline; 3 = t test - $p < 0.005$; 4 = Kruskal-Wallis test - $p < 0.005$.

beta-carotene, vitamin B12 and folate, though the results for these last two nutrients may have been influenced by the smaller number of subjects examined.

We also explored how supplementation influenced plasma nutrient levels by calculating the number of participants in the three lower quartiles at baseline who progressed at least one step on the quartile scale during follow-up. For GRAC, regression of at least one step for the three higher quartiles was calculated. Comparisons of the proportion of those assigned to placebo compared with those assigned to Centrum after one to two years of follow-up who increased by at least one quartile step are as follows: vitamin A (60% vs 48%); 95% CIs (52%-78% vs 41%-57%); vitamin E (66% vs 37%); 95% CIs (59%-75% vs 30%-45%); beta-carotene (76% vs 48%); 95%

CI (70%-84% vs 40%-56%); and vitamin C (68% vs 42%); 95% CIs (60%-75% vs 34%-50%). The proportion with improved riboflavin status as indicated by lower GRAC values for those assigned to placebo compared with those assigned to Centrum was: (87% vs 27%); 95% CIs (80%-92% vs 20%-35%).

By assuming that the upper quartile at baseline represented an “optimal” plasma value range we calculated the percent of participants who were included in this category for each nutrient after 1-2 years of supplementation. As shown in Table 3 after treatment with Centrum the percent of participants who were in the upper quartile of the baseline range was significantly increased over baseline for all nutrients, with the exception of vitamin A.

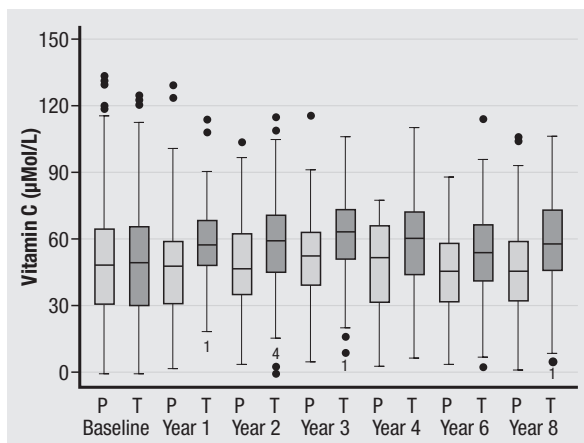


Fig. 5 | Baseline and follow-up mean levels of plasma vitamin C in placebo (P) and Centrum (T) treated subjects in CTNS. P vs. T change from baseline; 1 = t test - $p < 0.05$; 4 = Kruskal-Wallis test - $p < 0.005$.

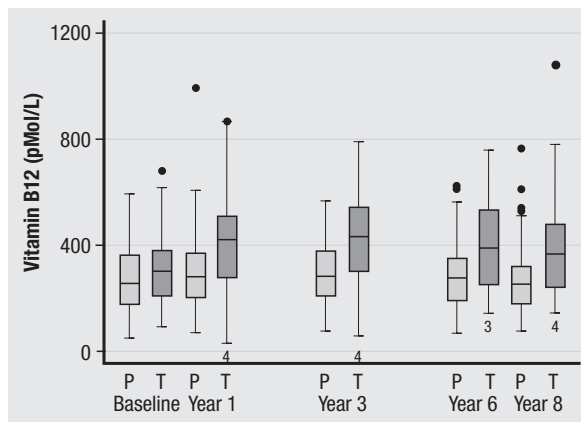


Fig. 7 | Baseline and follow-up mean levels of plasma vitamin B12* in placebo (P) and Centrum (T) treated subjects in CTNS. *Includes data for subset 1 only because plasma B12 levels were not measured at baseline in subset 2. P vs T change from baseline; 3 = t test - $p < 0.005$; 4 = Kruskal-Wallis test - $p < 0.005$.

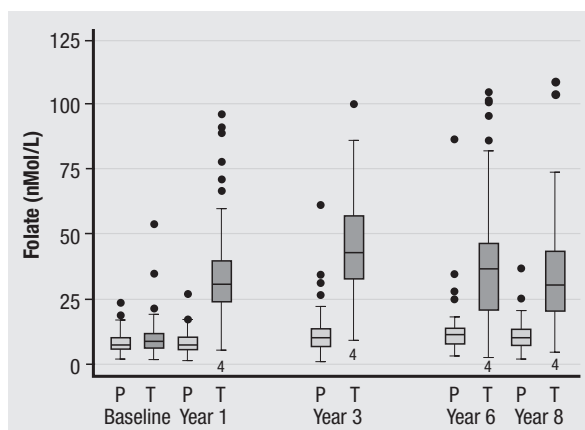


Fig. 8 | Baseline and follow-up mean levels of plasma folate* in placebo (P) and Centrum (T) treated subjects in CTNS. *Includes data for subset 1 only because plasma folate levels were not measured at baseline in subset 2. P vs T change from baseline; 4 = Kruskal-Wallis test - $p < 0.005$.

DISCUSSION

Mean nutrient plasma values of the CTNS cohort are similar to those reported in other western and Italian population samples [15, 17, 18]. The finding of higher plasma values of vitamin C and of beta-carotene in women is consistent with previous observations [15, 19-23]. We also found significantly higher ($p < 0.005$) plasma values of alpha-tocophe-

rol in women, in agreement with some but not all previous studies [15, 23-25]. The number of persons at risk of deficiency among free-living elderly people aged > 65 years in the UK and Northern Ireland was reported as 49% to 79% for riboflavin, 3-7% for folate, 15-17% for vitamin C, and 0% for vitamin B12 [21, 26]. These percentages are in reasonable agreement with those found in CTNS, with the exception of folate for which the numbers at risk of deficiency were higher. Baseline plasma values were not appreciably influenced in our sample by smoking history or alcohol use, though lower levels of alpha-tocopherol, beta-carotene and vitamin C have been reported in smokers [27, 28].

Our data indicate that supplementation with one daily tablet of Centrum®, a multivitamin/mineral supplement containing US RDI levels of nutrients, results in a significant increase in the mean/median plasma levels of most, but not all, of the nutrients tested in a cohort of well nourished home-living elderly people during a mean follow-up interval of 9 years. With respect to baseline values, plasma levels of vitamin E, beta-carotene, folate, and vitamin B12, and riboflavin status were significantly improved during follow-up compared with participants assigned to placebo, while differences were at a lower level of significance for vitamin C and borderline significant for vitamin A. Similar results were reported for vitamin C, E, and beta-carotene supplementation at dosages 1-3 times those present in Centrum in a six month, controlled trial of supplementation in women > 65 years in Germany [24] and in a larger

Table 3 | Centrum and placebo treated subjects with nutrient levels in the upper 25% of the total distribution at baseline after 1-2 years of follow-up in CTNS

Nutrient	Optimal value		Total No.	%	Confidence Interval
Vitamin A	2.43 µMol/L	Placebo	197	29.9	23.8 - 36.6
		Treatment	195	37.9	31.4 - 45.0
Vitamin E	41.26 µMol/L	Placebo	197	12.2	07.2 - 18.2
		Treatment	194	40.2	33.5 - 47.4
Beta-carotene	0.69 µMol/L	Placebo	194	27.8	21.8 - 34.4
		Treatment	183	63.9	57.2 - 71.3
Vitamin C	65.34 µMol/L	Placebo	197	19.3	14.0 - 25.1
		Treatment	195	32.8	26.5 - 39.7
Glutathione-reductase activation coefficient	1.196 UI/gHb*	Placebo	196	18.9	13.6 - 24.6
		Treatment	195	71.3	65.2 - 77.9
Vitamin B12	376.28 pMol/L	Placebo	90	21.1	13.1 - 31.1
		Treatment	91	54.9	43.9 - 65.9
Folate	11.33 nMol/L	Placebo	90	21.1	13.1 - 31.1
		Treatment	97	94.8	87.8 - 98.8

*The "optimal" value corresponds to the upper quartile at baseline for all nutrients except glutathione-reductase activation coefficient. For the glutathione-reductase activation coefficient the "optimal" value corresponds to the lower quartile.

randomized, double-blind, placebo controlled, primary-prevention trial with two years follow-up in France [30]. In an eight-week double-blind, placebo controlled clinical trial among 80 adults aged 50-87 years, supplementation with a multivitamin-mineral formulation similar to that used in our study significantly increased plasma concentrations of vitamin E, folate, and B12, but not vitamin A, and improved the riboflavin activity coefficient [31].

In our study the percentage of participants with plasma values above the accepted cut-point for risk of folate deficiency increased from 73% at baseline to 98% at the end of follow-up in those assigned to Centrum and from 58% to 87% in those assigned to placebo. Adequate riboflavin status can be estimated by the percentage of participants with GRAC <1.2. For those assigned to Centrum, the proportion with adequate riboflavin status increased from 75% at baseline to 96% during follow-up years one and two, but decreased again to a level similar to the baseline value at the 6th and 8th year follow-up visits, but still remained higher than this proportion in those assigned to placebo at these two visits but at borderline statistical significance ($p=0.052$ and 0.057 respectively) (data not shown).

We cannot explain the decreased effect of supplementation on riboflavin status over time as we have no evidence of a decreased compliance with the treatment regimen toward the end of the study. Madigan *et al.* (26) reported a favourable effect of riboflavin supplementation for three months in restoring adequate GRAC values in 40 elderly subjects at a dosage of 25 mg/day but, like Alexander *et al.* (32), found no effect at a dosage of 1.6 mg/day, which is similar to that present in one Centrum tablet.

Our results suggest that commonly used one-a-day multivitamin-mineral supplements formulated at about RDI levels can significantly raise the plasma levels of many of the nutrients included in such supplements. For several of the nutrients the effect seems greatest in those who start with lower plasma levels.

References

1. Radimer K, Bindewald B, Hughes J, Ervin B, Swanson C, & Picciano MF. Dietary supplement use by US adults: data from the National Health and Nutrition Examination Survey, 1999-2000. *Am J Epidemiol* 2004;160:339-49.
2. Timbo BB, Ross MP, McCarthy PV, Lin CT. Dietary supplements in a national survey: prevalence of use and reports of adverse events. *J Am Diet Assoc* 2006;106:1966-1974.
3. Rock CL. Multivitamin-multimineral supplements: who uses them? *Am J Clin Nutr* 2007;85:277S-9S.
4. Harrison RA, Holt D, Pattison DJ, Elton PJ. Are those in need taking dietary supplements? A survey of 21923 adults. *Brit J Nutr* 2004;91:617-23.
5. Schwarzpaul S, Strassburg A, Luhrmann PM, Neuhauser-Berthold M. Intake of vitamin and mineral supplements in an elderly German population. *Ann Nutr Metab* 2006;50:155-62.
6. Chen SY, Lin JR, Kao MD, Hang CM. The usage of dietary supplements among the elderly individuals in Taiwan. *Asia Pac J Clin Nutr* 2005;14:230-7.
7. Leske MC, Chylack LT, & Wu SY, The Lens Opacities Case-Control Study Group. The Lens Opacities Case-Control Study. Risk factors for cataract. *Arch Ophthalmol* 1991;109:244-51.
8. Mares-Perlman JA, Lyle BJ, Klein R, Fisher AI, Brady WE, VandenLangenberg GM, Trabulsi JM, Palta M. Vitamin supplement use and incident cataracts in a population-based study. *Arch Ophthalmol* 2000;118:1556-63.
9. Clarke R, & Armitage J. Antioxidant vitamins and risk of cardiovascular disease. Review of large scale randomized trials. *Cardiovasc Drugs Ther* 2002;16:411-5.
10. The CTNS Study Group. A randomized, double-masked, placebo-controlled clinical trial of multivitamin supplementation and age-related lens opacities. CTNS Report No 3. *Ophthalmology* 2008;115:99-607.

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11. The CTNS Study Group. The Italian-American Clinical Trial of Nutritional Supplements and Age-Related Cataract: design implications. CTNS report No. 1. *Controlled Clinical Trials* 2003;24:815-29.
12. Slimani N, Ferrari P, Ocké M, Welch A, Boeing H, van Liere M, Pala V, Amiano P, Lagiou A, *et al*. Standardization of the 24-hour diet recall calibration method used in the European prospective investigation into cancer and nutrition (EPIC): general concepts and preliminary results. *Eur J Clin Nutrition* 2000;54:900-17.
13. Gunter EW, Lewis BG, Koncikowski SM. *Laboratory procedures used for the third National Health and Nutrition Examination Survey (NHANES III) 1998-94*. Atlanta GA: National Center for Environmental Health, Centers for Disease Control and Prevention, Public Health Service, US Dpt of Health and Human Services; 1996.
14. Goldberg DM, Spooner RJ. Glutathione reductase. In: HU Bergmeyer (Ed). *Methods of enzymatic analysis*. New York: Academic Press; 1983. Vol 3, p. 258-65.
15. Palli D, Decarli A, Russo A, Cipriani F, Giacosa A, Amadori D, Salkeld R, Salvini S, Buiatti E. Plasma levels of antioxidant vitamins and cholesterol in a large population sample in central-northern Italy. *Eur J Nutr* 1999;38:90-8.
16. Russell RM, Suter PM. Vitamin requirements of elderly people: an update. *Am J Clin Nutr* 1993;58:4-14.
17. Valero MP, Fletcher AE, De Stavola BL, Vioque J, Alepuz VC. Vitamin C is associated with reduced risk of cataract in a Mediterranean population. *J Nutrition* 2002;132:1299-306.
18. Comstock GW, MS M, Schober SE, Vuilleumier JP, Helsing KJ. Serum levels of retinol, beta-carotene, alpha-tocopherol in older adults. *Am J Epidemiol* 1988;127:114-23.
19. Lentner C. Blood vitamins. In *Geigy Scientific Tables*. Basle: CIBA Geigy; 1984. p.125-34.
20. Wright AJA, Southon S, Bailey AL, Finglas PM, Maisey S, Fulcher RA. Nutrient intake and biochemical status in non-institutionalized elderly subjects in Norwich: comparison with younger adults and adolescents from the same general community. *Brit J Nutrition* 1995;4:453-75.
21. Bailey AL, Maisey S, Southon S, Wright AJA, Finglas PM, Fulcher RA. Relationship between micronutrient intake and biochemical indicators of nutrient adequacy in a "free-living" elderly UK population. *Brit J Nutrition* 1997;77:225-42.
22. Faure H, Preziosi P, Roussel AM, Bertrais S, Galan P, Hercberg S, Favier A. Factors influencing blood concentration of retinol, alpha-tocopherol, vitamin C, and beta-carotene in the French participants of the SU:VI:MAX trial. *Eur J Clin Nutrition* 2006;60:706-17.
23. Hallfrisch J, Muller DC, Singh VN. Vitamin A and E intakes and plasma concentrations of retinol, beta-carotene, alpha-tocopherol in men and women of the Baltimore Longitudinal Study of Aging. *Am J Clin Nutr* 1994;60:176-82.
24. Herbeth B, Chavance M, N. M, Mejean L, Vernhes G. Dietary intake and other determinants of blood vitamins in an elderly population. *Eur J Clin Nutr* 1989;47:175-86.
25. Hebert JR, Hurley TG, Hsieh J, Rogers E, Stoddard AM, Sorensen G, Nicolosi RG. Determinants of plasma vitamins and lipids: the Working Well Study. *Am J Epidemiol* 1994;40:132-47.
26. Madigan SM, Tracey F, McNulty H, Eaton-Evans J, Coulter J, McCartney H, Strain GG. Riboflavin and vitamin B-6 intakes and status and biochemical response to riboflavin supplementation in free-living elderly people. *Am J Clin Nutr* 1998;68:389-95.
27. Chow CK, Thacker RR, Changchit C, Bridges RB, Rehm SR, Humble J, Turbek J. Lower levels of vitamin C and carotenes in plasma of cigarette smokers. *J Am Coll Nutr* 1986;5:305-12.
28. Galan P, Viteri FE, Bertrais S, Czernichow S, Faure H, Arnaud J, Ruffieux D, Chenal S, Arnault N, *et al*. Serum concentrations of beta-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population. *Eur J Clin Nutrition* 2005;59:1181-90.
29. Wolters M, Hermann S, Hahn. Effects of 6-month multivitamin supplementation on serum concentrations of alpha-tocopherol, beta-carotene, and vitamin C in healthy elderly women. *Int J Vitam Nutr Res* 2004;74:161-8.
30. Malvy DJ, Favier A, Faure H, Preziosi P, Galan P, Arnaud J, Roussel AM, Briançon S, Hercberg S. Effect of two years' supplementation with natural antioxidants on vitamin and trace element status biomarkers: preliminary data of the SU.VI.MAX study. *Cancer Detect Prev* 2001;25:479-85.
31. McKay DL, Perrone G, Rasmussen H, Dallal G, Hartman W, Cao G, Prior RL, Roubenoff R, Blumberg JB. The effect of multivitamin-mineral supplement on micronutrient status, antioxidant capacity and cytokine production in healthy older adults consuming a fortified diet. *J Am Coll Nutr* 2000;19:613-21.
32. Alexander M, Emanuel G, Golin T, Pinto JT, Rivlin RS. Relation of riboflavin nutrition in healthy elderly to intake of calcium and vitamin supplements: evidence against riboflavin supplementation. *Am J Clin Nutr* 1984;39:540-46.

Supplemental Table 1 | Baseline values and change from baseline of plasma vitamin A (Retinol) levels ($\mu\text{Mol/L}$). Comparisons between placebo and Centrum treated subjects in CTNS

	PLACEBO							TREATMENT						
	Baseline	Year 1	Year 2	Year 3	Year 4	Year 6	Year 8	Baseline	Year 1	Year 2	Year 3	Year 4	Year 6	Year 8
N	509	96	101	96	95	147	126	510	98	97	96	90	144	137
Mean	2.06	2.16	2.20	2.27	2.16	2.23	2.69	2.06	2.27	2.37	2.37	2.34	2.41	2.76
St. Dev.	0.59	0.63	0.63	0.59	0.52	0.59	0.66	0.59	0.59	0.52	0.56	0.52	0.70	0.73
Median	1.99	2.16	2.20	2.23	2.13	2.20	2.62	2.02	2.16	2.37	2.37	2.27	2.34	2.58
CHANGE FROM BASELINE														
N		96	101	96	95	147	126		98	97	96	90	144	137
Mean		0.14	0.17	0.21	0.14	0.24	0.66		0.21	0.35 ¹	0.35	0.31 ¹	0.38	0.70
SE (Mean)		0.07	0.07	0.07	0.07	0.03	0.07		0.07	0.07	0.07	0.07	0.07	0.07
Median		0.10	0.21	0.17	0.14	0.17	0.59		0.28	0.35	0.38	0.31	0.35 ²	0.63

¹: t test - $p < 0.05$; ²: Kruskal-Wallis test - $p < 0.05$

Supplemental Table 2 | Baseline values and change from baseline of plasma vitamin E (α -tocopherol) levels ($\mu\text{Mol/L}$). Comparisons between placebo and Centrum treated subjects in CTNS

	PLACEBO							TREATMENT						
	Baseline	Year 1	Year 2	Year 3	Year 4	Year 6	Year 8	Baseline	Year 1	Year 2	Year 3	Year 4	Year 6	Year 8
N	508	96	101	96	95	147	127	508	97	97	95	90	143	137
Mean	34.69	32.62	32.69	31.81	30.67	32.13	36.73	36.10	39.96	40.98	40.12	37.91	38.49	42.65
St. Dev.	10.40	8.10	7.92	7.73	7.85	7.89	9.45	10.73	10.80	9.54	9.22	8.98	8.94	9.36
Median	32.81	32.74	31.74	31.13	29.167	31.46	35.66	34.38	38.19	39.77	40.91	36.96	37.33	42.14
CHANGE FROM BASELINE														
N		96	101	96	95	147	127		98	97	96	90	144	137
Mean		-1.14	-1.04	-1.93	-3.18	-1.63	3.41		4.62 ₃	3.71 ₃	4.97 ₃	0.65 ₁	2.23 ₃	5.99
SE (Mean)		0.88	0.81	1.02	0.88	0.88	0.93		1.11	1.30	1.09	1.25	0.97	0.97
Median		-0.58	0.28	-1.28	-1.90	-0.05	3.25		4.41	5.39	6.76	0.23	3.46	6.27

1 = t test - $p < 0.05$; 3 = t test - $p < 0.005$

Supplemental Table 3 | Baseline values and change from baseline of plasma beta-carotene levels ($\mu\text{Mol/L}$).
Comparisons between placebo and Centrum treated subjects in CTNS

	PLACEBO							TREATMENT						
	Baseline	Year 1	Year 2	Year 3	Year 4	Year 6	Year 8	Baseline	Year 1	Year 2	Year 3	Year 4	Year 6	Year 8
N	509	95	99	96	93	146	126	508	94	89	92	88	142	137
Mean	0.52	0.54	0.63	0.60	0.54	0.50	0.50	0.54	0.88	1.06	0.97	0.91	0.75	0.69
St. Dev.	0.35	0.37	0.45	0.47	0.35	0.34	0.35	0.35	0.47	0.60	0.48	0.52	0.45	0.47
Median	0.41	0.45	0.52	0.45	0.47	0.43	0.39	0.45	0.78	1.02	0.89	0.84	0.65	0.60
CHANGE FROM BASELINE														
N		95	99	96	93	146	126		94	89	92	87	141	136
Mean		0.05	0.13	0.13	0.04	0.02	0.01		0.35	0.55	0.42	0.35	0.20	0.11
SE (Mean)		0.04	0.04	0.04	0.04	0.02	0.04		0.04	0.06	0.06	0.04	0.04	0.0037
Median		0.05	0.09	0.05	0.02	0.02	-0.002		0.29 ₄	0.54 ₄	0.35 ₄	0.37 ₄	0.18 ₄	0.09 ₂

2 = Kruskal-Wallis test - $p < 0.05$; 4 = Kruskal-Wallis test - $p < 0.005$

Supplemental Table 4 | Baseline values and change from baseline of plasma vitamin C levels ($\mu\text{Mol/L}$).
Comparisons between placebo and Centrum treated subjects in CTNS

	PLACEBO							TREATMENT						
	Baseline	Year 1	Year 2	Year 3	Year 4	Year 6	Year 8	Baseline	Year 1	Year 2	Year 3	Year 4	Year 6	Year 8
N	504	96	101	96	95	142	118	506	98	97	96	90	137	129
Mean	49.11	47.01	49.17	50.59	48.77	46.22	46.84	49.96	58.54	59.05	63.08	59.56	53.09	59.10
St. Dev.	26.06	24.02	21.75	19.59	19.76	19.02	22.65	25.89	16.86	22.09	19.19	21.18	19.30	21.75
Median	48.37	47.69	46.84	52.92	51.89	45.88	46.16	49.79	57.68	59.39	63.53	60.69	53.88	58.14
CHANGE FROM BASELINE														
N		95	101	95	95	141	117		98	96	96	89	136	129
Mean		-0.40	5.05	3.41	4.26	-1.59	-1.02		8.23 ₁	12.43	12.43 ₁	12.09	2.84	8.63 ₁
SE (Mean)		2.55	2.21	2.38	2.50	2.04	2.44		3.01	2.55	3.12	2.78	2.61	2.50
Median		-1.02	4.66	4.14	7.10	0.51	0.40		10.73	14.65 ₄	13.51	8.35	0.79	6.59

1 = t test - $p < 0.05$; 4 = kruskal-wallis test - $p < 0.005$

Supplemental Table 5 | Baseline values and change from baseline of plasma glutathione-reductase activation coefficient. Comparisons between placebo and Centrum treated subjects in CTNS

	PLACEBO							TREATMENT						
	Baseline	Year 1	Year 2	Year 3	Year 4	Year 6	Year 8	Baseline	Year 1	Year 2	Year 3	Year 4	Year 6	Year 8
N	508	95	101	95	95	124	123	509	98	97	96	90	126	129
Mean	1.15	1.17	1.17	1.18	1.18	1.18	1.18	1.15	1.04	1.06	1.07	1.11	1.12	1.13
St. Dev.	0.11	0.11	0.13	0.13	0.15	0.16	0.15	0.11	0.04	0.08	0.07	0.13	0.15	0.15
Median	1.13	1.15	1.15	1.15	1.15	1.14	1.14	1.13	1.03	1.02	1.05	1.07	1.04	1.09
CHANGE FROM BASELINE														
N		94	101	94	95	125	123		98	97	96	90	126	129
Mean		0.03	0.02	0.03	0.03	0.04	0.04		-0.10 ₃	0.10 ₃	-0.08 ₃	-0.04	-0.04	-0.01
SE (Mean)		0.11	0.01	0.01	0.01	0.02	0.01		0.01	0.01	0.01	0.01	0.02	0.02
Median		0.03	0.04	0.03	0.04	0.01	0.01		-0.08	-0.09	-0.06	-0.05 ₄	-0.06 ₄	-0.04 ₄

3 = *t* test - $p < 0.005$; 4 = Kruskal-Wallis test - $p < 0.005$.

Supplemental Table 6 | Baseline values and change from baseline of plasma vitamin B12 levels (pMol/L).
Comparisons between placebo and Centrum treated subjects in CTNS

	PLACEBO							TREATMENT						
	Baseline	Year 1	Year 2	Year 3	Year 4	Year 6	Year 8	Baseline	Year 1	Year 2	Year 3	Year 4	Year 6	Year 8
N	99	95	101	91	94	145	125	104	91	95	90	87	140	132
Mean	276.60	293.50	335.55	297.55	328.32	299.55	289.37	305.74	412.43	449.54	425.49	450.94	392.44	393.84
St. Dev.	120.04	133.54	175.23	122.11	171.10	151.40	155.90	123.14	172.13	197.58	153.39	197.36	167.70	188.29
Median	250.85	280.36	287.74	295.12	287.37	264.13	244.21	302.50	420.55	405.79	431.61	435.30	363.37	355.25
CHANGE FROM BASELINE														
N		90		87		69	58		91		90		72	61
Mean		13.44		18.78		1.05	-5.94		100.44		117.63		79.76 ₃	71.71
SE (Mean)		12.11		9.86		10.79	14.02		13.77		13.35		15.52	20.69
Median		11.07		14.76		12.54	-5.90		88.54 ₄		114.36 ₄		50.91	51.56 ₄

3 = t test - $p < 0.005$; 4 = Kruskal-Wallis test - $p < 0.005$.

* Subset 1 only; no baseline measurement for subset 2.

Supplemental Table 7 | Baseline values and change from baseline of plasma folate levels (nMol/L).
Comparisons between placebo and Centrum treated subjects

	PLACEBO							TREATMENT						
	Baseline	Year 1	Year 2	Year 3	Year 4	Year 6	Year 8	Baseline	Year 1	Year 2	Year 3	Year 4	Year 6	Year 8
N	99	95	101	91	94	146	125	107	97	95	93	87	141	134
Mean	8.43	8.86	10.49	11.76	13.30	12.64	12.26	10.22	34.64	38.08	44.38	40.98	40.37	35.14
St. Dev.	3.92	4.33	5.51	7.93	10.85	8.45	6.59	6.39	18.12	21.16	19.96	23.13	24.99	23.22
Median	7.25	7.70	9.74	10.65	10.99	11.55	11.10	9.06	31.04	39.42	43.50	41.91	37.61	32.17
CHANGE FROM BASELINE														
N		90		87		70	58		97		93		72	63
Mean		0.25		3.19		3.99	2.76		24.54		34.28		29.93	25.08
SE (Mean)		0.35		0.77		1.22	0.70		1.95		1.99		2.88	2.83
Median		-0.45		1.59		2.38	1.59		21.30 ₄		34.66 ₄		28.55 ₄	22.88 ₄

4 = Kruskal-Wallis test - p < 0.005.

** Subset 1 only; no baseline measurement for subset 2.*

Supplemental Table 8 | Change from baseline of plasma vitamin A (Retinol) levels ($\mu\text{Mol/L}$) at year 1-2 by baseline quartiles in CTNS

	PLACEBO				TREATMENT			
	1 ST QUARTILE		4 TH QUARTILE		1 ST QUARTILE		4 TH QUARTILE	
	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2
N	116	47	128	48	137	53	126	44
Mean	1.33	1.82	2.86	2.69	1.33	2.02	2.86	2.72
St. Dev.	0.21	0.48	0.42	0.70	0.21	0.45	0.38	0.59
Median	1.36	1.71	2.76	2.65	1.36	1.99	2.72	2.76
CHANGE FROM BASELINE								
N		47		48		53		44
Mean		0.48		-0.17		0.70 ₁ **		-0.14 ₃ *
SE (Mean)		0.07		0.10		0.07		0.10
Median		0.42		-0.14 ₄ *		0.66		-0.21

1 = t test - p < 0.05; 3 = t test - p < 0.005; 4 = Kruskal-Wallis test - p < 0.005.
** Comparison of change in quartile 1 vs quartile 4 within treatment group.*
*** Comparison of change in quartiles between treatment groups.*

Supplemental Table 9 | Change from baseline of plasma vitamin E levels (α -tocopherol) ($\mu\text{Mol/L}$) at year 1-2 by baseline quartiles in CTNS

	PLACEBO				TREATMENT			
	1 ST QUARTILE		4 TH QUARTILE		1 ST QUARTILE		4 TH QUARTILE	
	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2
N	138	60	125	43	116	45	129	56
Mean	23.43	28.07	49.20	38.24	23.73	35.06	50.64	44.81
St. Dev.	3.41	7.10	6.78	8.10	2.74	7.82	7.73	12.33
Median	23.96	28.05	47.83	38.38	23.80	34.45	48.46	42.93
	CHANGE FROM BASELINE							
N		60		43		45		56
Mean		4.11		-10.10 [*]		11.05		-4.85
SE (Mean)		0.77		1.18		1.23		1.76
Median		3.71		-9.84		9.96 ^{**}		-7.20 ^{**}

2 = Kruskal-Wallis test - $p < 0.05$; 3 = t test - $p < 0.005$; 4 = Kruskal-Wallis test - $p < 0.00$.

* Comparison of change in quartile 1 vs quartile 4 within treatment group.

** Comparison of change in quartiles between treatment groups.

Supplemental Table 10 | Change from baseline of plasma beta-carotene levels ($\mu\text{Mol/L}$) at year 1-2 by baseline quartiles in CTNS

	PLACEBO				TREATMENT				
	1 ST QUARTILE		4 TH QUARTILE		1 ST QUARTILE		4 TH QUARTILE		
	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2	
N	129	51	126	44	125	43	128	43	
Mean	0.19	0.30	1.01	0.97	0.19	0.60	1.04	1.34	
St. Dev.	0.06	0.19	0.34	0.50	0.06	0.30	0.28	0.50	
Median	0.19	0.26	0.89	0.82	0.20	0.63	0.97	1.27	
CHANGE FROM BASELINE									
N		51		44		43		43	
Mean		0.13		² *	-0.04		0.39		0.34
SE (Mean)		0.02		0.06		0.04		0.09	
Median		0.07		-0.09		0.37 ₄ **		0.32 ₄ **	

² = Kruskal-Wallis test - $p < 0.05$; ₄ = Kruskal-Wallis test - $p < 0.005$.

* Comparison of change in quartile 1 vs quartile 4 within treatment group.

** Comparison of change in quartiles between treatment groups.

Supplemental Table 11 | Change from baseline of plasma vitamin C levels ($\mu\text{Mol/L}$) at year 1-2 by baseline quartiles in CTNS.

	PLACEBO				TREATMENT			
	1 ST QUARTILE		4 TH QUARTILE		1 ST QUARTILE		4 TH QUARTILE	
	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2
N	123	54	122	43	130	57	130	46
Mean	16.81	32.59	83.46	61.38	18.51	49.68	83.57	69.15
St. Dev.	8.46	21.35	17.15	18.91	8.40	19.30	15.16	20.61
Median	17.83	33.38	76.48	56.49	18.96	50.02	79.43	68.13
CHANGE FROM BASELINE								
N		54		43		57		46
Mean		15.67		-19.42		32.48 ₃ **		-16.52
SE (Mean)		2.61		3.58		2.61		3.97
Median		11.41		-17.43 [*]		32.08		-10.90 [*]

³ = *t* test - $p < 0.005$; ⁴ = Kruskal-Wallis test - $p < 0.005$.

^{*} Comparison of change in quartile 1 vs quartile 4 within treatment group.

^{**} Comparison of change in quartiles between treatment groups.

Supplemental Table 12 | *Change from baseline of plasma glutathione-reductase activation coefficient at year 1-2 by baseline quartiles in CTNS*

	PLACEBO				TREATMENT			
	1 ST QUARTILE		4 TH QUARTILE		1 ST QUARTILE		4 TH QUARTILE	
	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2	BASELINE	YEAR 1-2
N	130	56	126	45	125	52	126	54
Mean	1.04	1.09	1.31	1.27	1.04	1.03	1.30	1.08
St. Dev.	0.02	0.06	0.10	0.14	0.02	0.04	0.08	0.08
Median	1.04	1.10	1.29	1.24	1.04	1.02	1.28	1.06
	CHANGE FROM BASELINE							
N		56		45		52		54
Mean		0.06		-0.03		-0.004		-0.21
SE (Mean)		0.01		0.02		0.01		0.01
Median		0.06		-0.03 ₄ *		-0.01 ₄ **		-0.21 ₄ **

4 = Kruskal-Wallis test - $p < 0.005$.

* Comparison of change in quartile 1 vs quartile 4 within treatment group.

** Comparison of change in quartiles between treatment groups.

Supplemental Table 13 | Change from baseline of plasma vitamin B12 levels (pMol/L) at year 1-2 by baseline quartiles in CTNS

	PLACEBO				TREATMENT			
	1 ST QUARTILE		4 TH QUARTILE		1 ST QUARTILE		4 TH QUARTILE	
	BASELINE	YEAR 1	BASELINE	YEAR 1	BASELINE	YEAR 1	BASELINE	YEAR 1
N	31	28	21	20	20	16	26	24
Mean	149.77	208.13	458.84	429.40	145.35	244.88	468.65	541.84
St. Dev.	32.83	163.20	52.97	85.22	32.98	84.85	74.15	171.10
Median	154.94	180.76	457.44	424.24	147.56	228.72	450.06	509.08
CHANGE FROM BASELINE								
N		28		20		16		24
Mean		59.81		-29.14		103.29		75.93
SE (Mean)		29.47		23.42		21.17		35.42
Median		22.13		-22.13		48.85 ₄ **		47.96

2 = Kruskal-Wallis test - $p < 0.05$; 4 = Kruskal-Wallis test - $p < 0.005$.

* Comparison of change in quartile 1 vs quartile 4 within treatment group.

** Comparison of change in quartiles between treatment groups.

Supplemental Table 14 | Change from baseline of plasma folate levels (nMol/L) at year 1-2 by baseline quartiles in CTNS

	PLACEBO				TREATMENT			
	1 ST QUARTILE		4 TH QUARTILE		1 ST QUARTILE		4 TH QUARTILE	
	BASELINE	YEAR 1	BASELINE	YEAR 1	BASELINE	YEAR 1	BASELINE	YEAR 1
N	30	26	19	17	16	15	31	27
Mean	4.85	7.09	14.77	13.48	4.58	29.97	16.58	34.35
St. Dev.	1.04	3.15	3.19	4.96	1.13	12.12	8.56	12.91
Median	5.21	6.57	13.59	12.23	4.64	26.51	13.59	32.85
	CHANGE FROM BASELINE							
N		26		17		15		27
Mean		2.22		-0.93		25.40		17.90
SE (Mean)		0.61		1.20		3.01		2.99
Median		1.25		-2.04		21.30 ^{**}		18.35 ^{**}

4 = Kruskal-Wallis test - p < 0.005.
** Comparison of change in quartile 1 vs quartile 4 within treatment group.*
*** Comparison of change in quartiles between treatment groups.*