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Does Multivitamin Supplementation Prevent Infections in Healthy Elderly Subjects? A Controlled trial

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Summary: We performed a double blind randomized study in order to assess the efficacy of a multivitarnin supplement for the prevention of common infections in healthy elderly subjects. Non-institutionalized, independent subjects over 60 years of age, living in the Nevers area (central France) were recruited by announcements and randomly assigned to a treatment or a placebo group. The treatment or placebo tablets were to be taken daily for 4 months.

The principal criterion of response was the incidence of infections, as recorded from a detailed questionnaire presented at entrance, in the middle (2 months) and at the end (4 months) of the study. Blood samples were taken at the entrance in the study and after two and four months in order to monitor vitamin status.

. No significant difference was found between the two groups for the incidence of infections. In fact the observed incidences during the two two-month periods were higher in the treatment (0.38 and 0.21) than in the placebo group (0.29 and 0.13). After two months and after four months of supplementation, blood vitamin indicators of the subjects were significantly higher in the treatment than in the placebo group for vitamins B_t, 82, B_6, E and folates ($p< 0.001$). The improvement of the vitamin C status was slightly higher in the treatment than in the placebo group. For vitamin A status, the evolution was similar in the two groups.

In conclusion, short-term supplementation of healthy elderly subjects with the investigated supplement is not likely to afford a meaningful protection against common infections.

Introduction

It has been known for years that nutrition and especially vitamin status influences immunity [1-3]. In developing countries, vitamin A has been found to protect children against diarrhea and respiratory diseases [4], severity of infections [5], complications of measles [6, 7] and mortality ascribed to different types of infections [8], In industrialized countries, the elderly are the most likely to have their immunity marginally impaired by marginal nutritional deficiencies. For this reason, and because of the decline of immunity with age [9], they also are the most likely to have their immunity enhanced by nutritional supplementation. Although epidemiological surveys failed to demonstrate convincing relations between vitamin status and impairment of immunity in healthy elderly subjects [10-12], one of them found a negative relationship between experience of infections and vitamin E status [11]. Besides, the main motive for supplementing elderly subjects should be direct clinical benefit and not mere improvement of immunological indices. For instance does vitamin supplementation decrease the incidence of infections? This question has been investigated for vitamin C and common colds; W.R. Beisel, reviewing the trials, con-

eluded that 1-2 g/d of ascorbic acid supplements appeared to be ineffective for the prevention of common colds [3]. From a pragmatic point of view, multivitamin supplements are more widely used than single vitamin supplements. Thus we chose to investigate the effect of multivitamin supplementation on the incidence of common infections in a population of healthy elderly subjects.

Subjects and Methods

This survey was performed in the Nevers area (central France), from January to December 1989. The subjects were volunteers, recruited by an announcement in the newspaper, lectures in senior citizens' clubs, or collaboration of general practitioners. They had to be independent, over 60 years of age, without any known disease or condition which might modify their nutritional status, and they should not have used any vitamin supplementation for three months prior to entrance in the study. The trial was presented as a study of the effect of a multivitamin supplement on health and the subjects knew that the treatment they would receive might not be efficient. Among 238 subjects who were informed of the objectives and of the protocol of the study, 218 gave oral consent and were eligible for the study. Their general practitioners were also informed. They were randomly assigned either to the treatment (n = 110) or to the placebo group (n = 108) according to a sampling scheme insuring balance within groups of 6 consecutive subjects.

The supplement, Azedavit®, combined hydrosoluble and liposoluble vitamins, oligoelements and minerals. Each tablet included 5,0001.U. of vitamin A (retinol acetate), 400 111 of vitamin Dz (ergocalciferol), 30 I.U. of vitamin E (dl a-tocopherol acetate), 90 mg of ascorbic acid, 2.25 mg of thiamin, 2.6 mg of riboflavin, 3 mg of pyridoxine, 9 |o,g of cyanocobalamin, 0.4 mg of folic acid, 20 mg of nicotinamid, 45 (j.g of biotin, 10 mg of pantothenic acid, 162 mg of calcium, 125 mg of phosphorus, 150 (j.g of iodine, 27 mg of iron, 100 mg of magnesium, 3 mg of copper, 7.5 mg of manganese, 7.5 mg of potassium, and 22.5 mg of zinc. The treatment and the placebo tablets were kindly provided and randomized by «Laboratoires Lederle», Rungis; they had the same appearence and were to be taken daily for four months.

At the entrance into the study (to), a detailed questionnaire about experience of infections during the last three years was presented; it included 14 headings dealing with diagnosis or symptoms of respiratory, nose, throat, ear, skin, mouth, urinary and gynecologic infections. For instance *during the last three years did you ever have a cold (consider it a cold only if it lasted more than 3 days)? if yes how many times? did you have it during the last month? did your general practitioner tell you it was the flu? during the last three years, did you ever have the flu? if yes, how many times? did you have the flu during the last month?* . . . The questionnaire investigated also socioeconomic characteristics. A clinical examination was performed and blood specimens were collected. The same questionnaire, restricted to the last two months, was presented and blood was collected again two months later (t:) and at the end of the study, four months later (t_4). A new supply of tablets was given at t2. Unused tablets were counted at *12* and 14 to asses compliance. A phone call was made or a short visit paid to the subjects one month and three months after entrance, in order to prevent withdrawal.

Dietary intake was assessed at to and 14 from food records collected over 7 successive days. All food items and quantities were recorded on standard forms. At the end of the 7-day recording period, a trained dietitian visited the subjects at home to collect the diet records and review them for completeness and accuracy. All food records were analyzed after coding, using a computerized version of the McCance and Widdowson dietary composition tables [13].

Plasma concentrations of a-tocopherol and retinol were determined with HPLC [14], and plasma ascorbic acid by a fluorometric method [15]. Plasma and erythrocyte folate were measured by the lactobacillus casei method [16]. Thiamin, riboflavin and vitamin B_6 status were assessed by measuring the coenzyme stimulation of vitamin dependent enzymes in erythrocytes: transkelotase (ETK), glutathione reductase (EGR) and glutamate oxaloacetate aminotransferase (EGOT) respectively [17]; low values are indicative of a good vitamin status. Some blood samples were lost or thawed during transportation and the corresponding measurements are missing.

Sample size insured a statistical power 1-(3 = 0.95 for a one-sided test of size a = 0.05 if the 4-month incidence was reduced from 0.6 to 0.4, assuming a standard deviation a = 0.75. Statistical comparisons were performed using two-sided t-tests (unless specified) and analysis of variance programs from the BMDP® statistical software.

Results

In each group, 7 subjects (6.5%) withdrew from the trial before its end. Five withdrawals were due to medical reasons, three in the treatment group (two digestive problems, one street accident) and two in the placebo group (one digestive problem, one cardiac insufficiency), the other withdrawals were due to non-compliance or impossibility to be present at appointments. The remaining subjects in the treatment and the placebo group were similar in terms of age (72.6±6.8 vs 72.4±6.3, mean±sd), sex (32 men and 71 women vs 37 men and 64 women), body mass index (25.5±4.6 vs 25.2±3.6), smoking history (7 vs 3 smokers), experience of infections during the last 3 years (1.96±2.47 vs 2.08 ±2.80) as well as for socioeconomics status

Table I: Socioeconomic status of the subjects in the treatment and placebo groups

	treatment	placebo
farmers	1	3
craftsmen	14	6
white collars	57	67
blue collars	10	7
no profession	13	8
information missing	8	10

(Tab. I), nutrient intake (Tab. II), medical history and medication use (data not shown).

Table III gives the vitamin status of the subjects at to, t2 and t4 for vitamins A, E, C, BI, B_2, Be and folates. At the entrance in the study, no significant difference was observed between the treatment and the placebo groups for the performed measurements. Vitamin blood indicators were significantly improved in the treatment as compared to the placebo group for vitamins E, BI, B_2, B_6 and folates at t_2 ($p<0.001$) and t4 ($p < 0.001$). The increase of plasma ascorbic acid concentration 4 months after entrance in the study was only slightly higher in the treatment than in the placebo group ($p<0.05$, one-sided test). The evolution of the concentrations of plasma retinol was similar in the two groups. Table IV gives the number of infections experienced during the last three years and observed during the study. Neither past experience nor incidence of common infections were significantly different in the two groups. Moreover, greater incidences of infections were recorded during each of the two-month periods in the treatment (0.38±0.64 and 0.21±0.57) than in the placebo group (0.29±0.59 and 0.13 ±0.37). The 90% symmetric confidence interval for the difference between the number of infections in the treated and in the placebo groups during the whole study was [-0.02,

Table II: Daily nutrients intake at baseline in placebo and treatment groups

	Treatment	Placebo
Energy (MJ/d)	8.11 ± 2.25*	8.07 ± 1.96
Protein (%)[1]	17.3 ± 2.9	17.6 ± 2.6
Carbohydrate (%)	44.6 ± 6.8	44.8 ± 6.2
Fat (%)	38.2 ± 6.4	37.6 ± 6.1
Vitamin A (µRE/d)[2]	1459 ± 1300	1301 ± 946
(µRE/5MJ)[3]	968 ± 982	830 ± 631
Vitamin E (mg TE/d)[4]	11.1 ± 5.9	11.4 ± 5.7
(mg TE/5MJ)	7.0 ± 3.8	7.1 ± 2.9
Vitamin C (mg/d)	80.5 ± 38.7	79.0 ± 35.9
(mg/5MJ)	51.9 ± 26.4	51.2 ± 25.8
Folic acid (µg/d)	201 ± 63	202 ± 67
(µg/5MJ)	128 ± 39	127 ± 37
Vitamin B_{12} (µg/d)	7.56 ± 6.81	7.23 ± 4.78
(µg/5MJ)	5.00 ± 5.11	4.57 ± 3.12
Vitamin B_1 (mg/d)	1.11 ± 1.03	0.98 ± 0.44
(mg/5MJ)	0.68 ± 0.47	0.61 ± 0.20
Vitamin B_2 (mg/d)	1.66 ± 0.50	1.62 ± 0.50
(mg/5MJ)	1.06 ± 0.34	1.02 ± 0.26
Vitamin B_6 (mg/d)	1.31 ± 0.36	1.36 ± 0.39
(mg/5MJ)	0.83 ± 0.17	0.85 ± 0.15

* mean ± sd
1 in percent of non alcohol energy
2 RE = retinol equivalent
3 nutrient density
4 TE = tocopherol equivalent

Table III: Evolution of blood vitamin indicators in the treatment and placebo groups

	group	n	mean ± sd t_0*	t_2[1]	t_4[2]
plasma α-tocopherol mg/l	placebo	95	13.4 ± 2.5	14.2 ± 2.5	14.7 ± 2.6
	treatment	83	13.7 ± 2.9	16.2 ± 2.8[3]	16.2 ± 2.6[3]
plasma retinol µg/l	placebo	99	615 ± 168	636 ± 164	646 ± 172
	treatment	88	602 ± 154	625 ± 151	624 ± 147
plasma ascorbic acid mg/l	placebo	76	6.05 ± 3.31	7.14 ± 5.07	8.23 ± 4.78
	treatment	88	5.29 ± 3.14	7.14 ± 3.16	8.98 ± 5.89[4]
plasma folates µg/l	placebo	100	7.53 ± 4.07	8.86 ± 5.65	8.66 ± 4.50
	treatment	98	7.04 ± 4.51	21.76 ± 11.31[3]	26.67 ± 11.75[3]
erythrocyte folates µg/l	placebo	96	300 ± 124	318 ± 176	352 ± 189
	treatment	91	290 ± 132	640 ± 311[3]	1042 ± 724[3]
B_1 status α ETK[5]	placebo	99	1.13 ± 0.09	1.14 ± 0.10	1.12 ± 0.08
	treatment	96	1.14 ± 0.10	1.07 ± 0.08[3]	1.07 ± 0.06[3]
B_2 status α EGR[5]	placebo	99	1.36 ± 0.17	1.24 ± 0.22	1.18 ± 0.12
	treatment	96	1.26 ± 0.17	1.11 ± 0.09[3]	1.09 ± 0.08[3]
B_6 status α EGOT[5]	placebo	99	1.78 ± 0.23	1.76 ± 0.21	1.74 ± 0.23
	treatment	96	1.78 ± 0.29	1.53 ± 0.16[3]	1.56 ± 0.19[3]

* entrance in the study
1 entrance + 2 months
2 entrance + 4 months
3 $p < 10^{-3}$ for comparison of the differences t_2-t_0 or t_4-t_0 in the placebo and treatment groups by t-test
4 $p < 0.10$ for comparison of the differences t_4-t_0 in the placebo and treatment groups by t-test
5 activation coefficient = activity after coenzyme saturation/basal activity

Table IV: Number of infections in the treatment and placebo groups

	treatment n = 103	placebo n = 101
last 3 years	1.96 ± 2.47*	2.08 ± 2.80
first 2 months	0.38 ± 0.64	0.29 ± 0.59
last 2 months	0.21 ± 0.57	0.13 ± 0.37

* mean ± standard deviation

+ 0.37]. Results were not essentially different if a square root transformation was applied, as recommanded for Poisson-type distributions or if analysis was restricted to current non smokers (data not shown). If subjects were separated in 3 subsamples, according to period of follow-up, the observed incidence of infections during the second two-month period was always greater in the treatment than in the placebo group. The observed incidence during the first two-month period was greater in the treatment than in the placebo group for 2 of the 3 subsamples (Tab. V).

Discussion

Does short term multivitamin supplementation prevent infections in healthy elderly subjects? As far as we know, this is the first trial investigating this question. In spite of the differences of evolution of the vitamins E, C, BI, Bz, B_6 and folic acid blood indicators in the two groups, the incidence of infections was not significantly different in the treatment and in the placebo group. The observed difference (+ 0.17) was not in the expected direction, and the lower bound of the *90%* symmetric confidence interval for the difference between the 4-month incidence in the two groups was only -0.02. In other words, if the true difference was -0.02 or less, the chances of observing a positive difference as great as 0.17 would be smaller than 5%, thus even if supplementation had some effect, the treatment of 100 subjects would not be likely to prevent more than two common infections in four months; quite a small clinical benefit. Several elements could contribute to the discrepancy between this survey and several animal or experimental studies.

Table V: Number of infections in the treatment and placebo groups according to period of follow up

Period of follow up		1*	2[1]	3[2]
First 2 months	Treatment	0.51 ± 0.74[3] (43)[4]	0.22 ± 0.49 (32)	0.36 ± 0.62 (28)
	Placebo	0.34 ± 0.68 (35)	0.32 ± 0.64 (34)	0.19 ± 0.40 (32)
Last 2 months	Treatment	0.21 ± 0.60 (43)	0.16 ± 0.57 (32)	0.29 ± 0.53 (28)
	Placebo	0.09 ± 0.37 (35)	0.15 ± 0.36 (34)	0.16 ± 0.37 (32)

* entrance in winter
1 follow up in spring and summer
2 entrance in summer, last visit in november or december
3 mean ± sd
4 number

Animal experiments establishing the existence of relations between nutrition and immunity generally imply severe deprivation or supplementation with relative doses quite larger than those used in this study. For vitamin C, for instance, many studies were performed on scorbutic guinea pigs [18]. Obviously, no sign of scurvy was observed in our subjects or in those studied in the trials of common cold prevention by vitamin C supplementation. Vitamin E supplementation was found to protect different species of laboratory as well as farm animals [19, 20]. In humans, supplementation of vitamin E neither alone [21] nor in association with other nutrients in this trial, led to a significantly decreased incidence of infections. Among the numerous elements that can contribute to this contradiction (species differences, animals' stress, ...) one should underline the fact that most animal studies relied on experimental infections. According to Tengerdy, «vitamin E is most beneficial in infectious diseases, mostly bacterial, where immune phagocytosis is the chief defensive mechanism » [19]. HARMAN and MILLER'S trial and ours investigated the incidence of all types of infections, bacterial as well as viral. But from a pragmatic point of view, they provided an estimate of the global benefit that could be expected from vitamin E supplementation.

Human studies reporting a protective effect of vitamin A were all performed in children at risk of vitamin A deficiency [4-8]. None of our subjects was classified in the high risk group (plasma retinol concentration < 287 |ig/l) or in the medium risk group (287 < plasma retinol concentration <401 M.g/1). The evolution of plasma retinol concentration was similar in the two groups, an observation that goes along with adequate hepatic stores in the studied population.

Improvement of immune indices have been observed in several controlled trials investigating the effect of single vitamin supplementation, but some of these results have not been duplicated yet [22]. Besides, a modification of some biological measurements does not necessarily imply a clinical benefit. For instance delayed-type hypersensitivity and lymphocyte proliferation in response to different mitogens have been found enhanced after vitamin C supplementation [23, 24], although such a supplementation does not seem to protect against the common cold [3].

Slightly different results might have been observed if the whole trial had taken place during fall and winter, when respiratory infections are most frequent. Statistical power could have been increased, but use of supplements is not generally restricted to any season. In any case, observations performed during the «bad season» (first two month-period for subjects who entered the study in winter, second period for those last seen in November or December) are consistent with the other observations.

Subjects were supplemented for four months only. Results might also have been different if we had studied the effect of long term sup-

plementation. GOODWIN and GARRY were able to investigate the effect of long term vitamin supplementation on the immunity of healthy elderly subjects in a cross-sectional epidemiological survey [10], but they did not report any dramatic effect of supplementation and concluded that the few significant differences «could have been due to chance».

In conclusion, this study does not support the hypothesis that short-term multivitamin supplementation of healthy elderly subjects might prevent a meaningful number of infections.

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