ELSEVIER

Nutrition Research 22 (2002) 5–11

NUTRITION RESEARCH

www.elsevier.com/locate/nutres

Influence of multinutrient supplement on immune responses and infection-related illness in 50–65 year old individuals

R.K. Chandra*

Privat Hospital, Gurgaon, India, and the Health Sciences Centre, Room 2J740, St. John's, NF A1B 3V6, Canada

Received 15 October 2001; accepted 15 October 2001

Abstract

The effect of a micronutrient supplement containing physiological amounts of trace-elements and vitamins on selected immune responses and infection was evaluated in 44 apparently healthy subjects aged 50–65 years for 12 months. At the baseline, 19 out of 44 (43.2%) subjects showed evidence of one or more nutrient deficiencies. In the supplemented group, all individuals except one showed normal nutrient levels when tested at 6 months; one person with iron deficiency persisting at 6 months required another 6 months of therapy and showed normal iron levels at 12 months. In the control group, there was no change in the prevalence of nutrient deficiencies. There was a significantly higher antibody response to influenza virus vaccine and an increase in the number of T lymphocytes and CD4+ helper T cells in the supplemented group compared with the placebo group. Interleukin-2 production was higher in the treated individuals compared with controls. Among the supplemented group, the immune responses increased more in those with one or more nutrient deficiencies compared with those with normal nutrient levels. The total number of days of infection during the one year of observation was 11.1 ± 0.8 in the supplemented group and 23.7 ± 2.1 in the placebo group ($p<0.01$). The beneficial effect on morbidity was of greater magnitude during the 7–12 month period of the trial. It is concluded that supplementation with modest amounts of vitamins and trace-elements results in higher immune responses and lower incidence of infection in the 50–65 year age group.

* Corresponding author. Janeway Child Health Centre, Room 2J740, St. John's NF A1B 3V6, Canada. Tel.: +1-709-777-4519; fax: +1-709-753-3322.

E-mail address: rchandra@mun.ca (R.K. Chandra).

0271-5317/02/$ – see front matter
PII: S0271-5317(01)00366-9

Keywords: Older subjects; Influenza vaccine; Cell-mediated immunity; T lymphocytes; Infection; Vitamins; Trace-elements

1. Introduction

Many studies have demonstrated an age-related decline in immune functions and an increased incidence of infection [1–3]. These two phenomena may be causally related. It is now established that nutritional status is a critical determinant of immunity in all age groups [4–8]. A few studies have examined the effect of nutrient supplementation on immune response of elderly subjects above 65 years of age [9–13]. Some have used a single nutrient whereas others employed a multinutrient supplement. In this study, we looked at the impact of supplementation with modest amounts of all essential vitamins and trace-elements on selected immune responses and infection-associated illness in a group of healthy 50–65 year old subjects.

2. Subjects and methods

The basic design of the study was similar to a previously published investigation and reference should be made to those publications [14,15]. The 44 subjects were aged 50–65 years; there were 22 males and 22 females. Three subjects (two men, one woman; all from the placebo group) dropped out of the study for personal reasons. The observation period was 12 months.

The composition of the daily oral supplement was similar to that used in the earlier study [14,15]. It contained vitamin A 400 retinol equivalents, beta-carotene 16 mg, thiamin 2.2 mg, riboflavin 1.5 mg, niacin 16 mg, vitamin B6 3.0 mg, folate 400 mcg, vitamin B12 4.0 mcg, vitamin C 80 mg, vitamin D 4.0 mcg, vitamin E 44 mg, iron 16 mg, zinc 14 mg, copper 1.4 mg, selenium 20 mcg, iodine 200 mcg, calcium 200 mg, magnesium 100 mg. The placebo contained calcium 200 mg and magnesium 100 mg.

Blood samples were obtained twice at one-week interval prior to supplementation and again at the end of 6 and 12 months. These were analyzed for levels of selected nutrients and for defined immune responses as described earlier [14]. The average of three estimations on the two blood samples taken one-week apart at the beginning and at the end of the 6 months and 12 months was used for the analyses.

One dose of influenza virus vaccine A/Sydney and A/Beijing was given approximately at the end of 3 months of the study; blood sample for antibody levels was drawn 12 weeks later. Antibody titre was determined by hemagglutinin-inhibition assay. Log10 HI antibody titres for A/Beijing are reported.

Infection was diagnosed based on clinical history of fever, cough, elevated sedimentation rate and C-reactive protein, X-ray of the sinuses and chest, blood culture, sputum culture, and urine culture. Each subject was contacted at least once in two weeks to obtained history of illness.

Table 1
Effect of multinutrient supplementation on immune responses

Parameter	Placebo			Supplement		
	0m	6m	12m	0m	6m	12m
Number of subjects	22	19	19	22	22	22
T lymphocytes (%)						
Mean	56.3	59.1*	58.6	59.3	68.1*	69.7
SD	4.1	2.5	3.4	2.7	2.9	2.3
CD4+ cells						
Mean	40.1	38.6*	40.5	41.3	49.3*	50.5
SD	2.3	2.7	2.6	3.4	3.1	2.9
Interleukin-2 (U/ml)						
Mean	3.8	4.0**	3.6	4.6	10.8**	11.7
SD	0.5	0.4	0.7	1.0	0.6	0.9
HI antibody titre (geometric mean)	27	69**	ND	19	157**	ND

Statistical significance of differences; * <0.05, ** <0.02.
ND = not done
0m = At baseline, 6m = At 6 months of the study, 12m = at the end of 12 months.

The overt prevalence of nutrient deficiencies at 0 and 6 months was compared by the Fisher's exact probability test. The two groups were compared with respect to mean change in immune responses by ANOVA. Results of nutrient levels and immune responses were correlated by Pearson's coefficient. Wilcoxon rank-sum test was used where appropriate. SPSS and minitab software packages were used to analyse results.

3. Results

The immunologic results are shown in Table 1. In the supplemented group, there was a significant increase in the number of T lymphocytes, CD4 cells, and IL-2 production, both at 6 and at 12 months. The enhancement in the number of cells and IL-2 production was observed by 6 months of the study; no further significant increase was seen between 6 and

Table 2
Number of subjects and the overall prevalence of nutrient deficiencies

	Placebo			Supplement		
	0m	6m	12m	0m	6m	12m
Number of subjects	22	19	19	22	22	22
Male/female	7/15	5/14	5/14	7/15	7/15	7/15
Deficiency of one or more nutrients (%)	40.9	47.4	52.6	45.4	4.5	0

Subjects whose blood nutrient levels were below the 95% confidence limits of the "normal reference standard" [15] were defined as "deficient."
0m = At baseline, 6m = at 6 months of the study, 12m = at the end of 12 months.

Table 3
Percentage prevalence of nutrient deficiencies

Nutrient	Placebo			Supplement		
	0m	6m	12m	0m	6m	12m
Vitamin A	4.5	5.3	9.1	4.5	0	0
Beta-carotene	13.6	15.8	15.8	9.1	0	0
Vitamin B6	13.6	10.5	10.5	13.6	0	0
Folic acid	9.1	10.5	15.8	9.1	0	0
Vitamin B12	9.1	10.5	10.5	9.1	0	0
Vitamin C	9.1	15.8	21.1	13.6	0	0
Vitamin D	4.5	5.3	5.3	9.1	0	0
Vitamin E	4.5	10.5	10.5	9.1	0	0
Iron	13.6	15.2	10.5	13.6	4.5	0
Zinc	13.6	15.2	15.2	13.6	0	0

Subjects whose blood nutrient levels were below the 95% confidence limits of the "normal reference standard" [15] were defined as "deficient."

0m = at baseline, 6m = at 6 months of the study, 12m = at the end of 12 months.

12 months. Antibody level to influenza virus at 6 months was higher in the supplemented individuals than in placebo controls (Table 1).

At the beginning of the trial, 19 of 44 subjects, or 43.2 percent, showed evidence of deficiency of one or more nutrients. There was no difference in the prevalence of nutrient deficiencies between the two groups (Table 2).

By the end of 6 months, there was a significant increase in the blood nutrient levels in supplemented persons, resulting in a reduced prevalence of nutrient deficiency (Table 3). By the end of 12 months, only one subject showed evidence of iron deficiency. On the other hand, in the placebo group, there was no significant change in the prevalence of deficiency of any nutrient at both 6 and 12 months of observation.

Infection was experienced significantly less often in the supplemented subjects compared with controls, both during the 0–6 and 7–12 month periods of the study (Table 4). The differences between the two groups were more significant in the 7–12 month period of the study. This resulted in a very significant reduction in the total number of days of illness with infection during the entire period of the one-year study. In the supplemented group, the reduction in 6-month morbidity was greater in the second half of the trial compared with the first half.

Table 4
Infection-related illness

Group	Morbidity (days)		
	0–6 months	6–12 months	0–12 months
Placebo controls	12.7 ± 1.6*	11.0 ± 1.2***	23.7 ± 2.3**
Supplemented	8.5 ± 0.7*	2.6 ± 0.3***	11.1 ± 0.8**

Statistical significance of differences: * <0.5, ** <0.02, *** <0.004.

Data are shown as Mean ± SD.

Table 5
Effect of supplementation on immune responses in those with one or more nutrient deficiencies at the baseline and in those with no nutrient deficiency

Parameter	Deficient (10)		Normal nutrient levels (n = 12)	
	0m	6m	0m	6m
T lymphocytes (%)	54.5 ± 3.8	65.3 ± 5.1**	61.6 ± 5.7	68.2 ± 4.6*
CD4+ T cells (%)	40.6 ± 3.0	52.1 ± 2.7**	44.5 ± 3.2	51.4 ± 3.8*
Interleukin-2 (U/ml)	2.9 ± 0.5	9.5 ± 1.1***	8.0 ± 0.9	11.9 ± 1.2*
Antibody response to influenza virus (geometric mean)	22	168***	56	153**

0m = at the baseline, 6m = at 6 months of the study.
Data are shown as Mean ± SD.
Statistical difference of 6 month response and 0 month response within the "deficient" or "adequate" groups. * = <0.05, ** = <0.02, *** = <0.007.

Multiple regression analyses showed that no single nutrient was responsible for the change in immune responses in the supplemented group. All supplemented individuals showed higher response on one or more immune function tests. However, those with evidence of nutrient deficiencies at the beginning of the trial showed a bigger improvement in immunity compared with that seen in those with no nutrient deficiency at the onset (Table 5).

There were no side effects that could be attributed to the use of the micronutrient supplement.

4. Discussion

Previous studies have attested to the role of nutrient deficiency as a factor in modulating immunity and risk of infection in the elderly above 65 years of age. Some studies showed improvement in a few immunologic parameters with the use of a single nutrient supplement, such as zinc, vitamin B6, others. However, such trials did not examine or did not show any beneficial effect on infection-related morbidity.

The rationale for using a multinutrient supplement to improve immune responses has been discussed [14]. At least 40 percent of the elderly have low blood levels of one or more nutrients. Multiple nutrient deficiencies are seen. At the same time, it is impractical and extremely costly to attempt dietary intake and blood analyses for all the important nutrients on all individuals in the populations. Furthermore, modest almost physiological amounts of vitamins and trace-elements have been used for prolonged periods of months and years by apparently healthy individuals and no side-effects were observed. In the light of these observations, it was pertinent to conduct a prospective randomized placebo-controlled study in which all essentially vitamins and trace-elements were administered daily; besides immune responses, infection-related morbidity was observed [15]. From the results of this trial, it was concluded that the daily administration of a supplement containing modest amounts of vitamins and trace elements resulted in an increase in the number of T cell subsets, natural

killer cells, and an increase in natural killer cell activity, interleukin production and antibody response to influenza virus vaccine. There was a marked reduction in infection-related illnesses, both mild and severe. This resulted in lesser need for antibiotic usage.

These results have been confirmed and extended in other studies. For example, Jain [16] used the same supplement of vitamins and trace elements and showed similar benefit in terms of reduction in respiratory illness. In very old elderly residents of long-stay institutions with an average age of 85 years, the daily use of the same supplement for 19 months was associated with fewer days of illness in the later part of the study, namely from 4–19 months (Allard JA, 2001, personal communication). This study suggests that in the very frail old elderly, it might take up to 3 months for such a supplement to show a clinical benefit in terms of reduced infection-related morbidity.

Preliminary calculations indicate that such a supplement would cost approximately US$10 per month and would result in a saving of at least $280 in fewer visits to doctors, less expense on laboratory tests such as blood examination and radiology, less need for antibiotics and other prescription and over-the-counter medications, and reduced number of days of hospitalization. In addition, there is clearly a reduction in morbidity, fewer days of absenteeism from work that would translate into greater productivity. All this adds to an extremely cost-benefit and cost-effective intervention.

The present study confirms and extends the observations of the earlier study [15] and shows that the daily administration of the same supplement of micronutrients results in similar benefits for immunity and risk of infection for the slightly younger age group of 50–65 years. The benefits were demonstrated within 6 months of the supplementation. No side effects from the use of the supplement were noted.

It is concluded that nutrient deficiency observed in the age group of 50–65 years is associated with impaired immunity that can be restored to normal within 6 months by providing a supplement containing modest amounts of all essential vitamins and trace elements. These findings have considerable significance for maintaining health and prevention of disease. The intervention is both cost-beneficial and cost-effective.

References

[1] Roberts-Thompson IC, Wittingham S, Young-chaiyud U, Mackey IR. Ageing, immune response, and mortality. Lncet 1974;2:368–70.
[2] Chandra RK. Nutrition, immunity and illness in the elderly. New York: Pergamon Press, 1984.
[3] Wayne SJ, Rhyne RL, Garry PJ, Goodwin JS. Cell-mediated immunity as a predictor of morbidity and mortality in the aged. J Gerontol Soc 1990;45:M45–M48.
[4] Chandra RK, Newberne PM. Nutrition, immunity and infection;mechanisms of interactions. New York: Plenum Press, 1977.
[5] Gershwin ME, Beach RS, Hurley LS. Nutrition and immunity. New York: Academic Press, 1984.
[6] Chandra RK. Nutrition, immunity and infection. Proc Natl Acad Sci USA 1996;93:14304–7.
[7] Suskind RM, Tontisirin K, editors. Nutrition, immunity and infection in infants and children. Philadelphia: Lippincott Williams & Wilkins, 2001.
[8] Duchateau J, Delepesse G, Vrijens R, Collett H. Beneficial effects of oral zinc supplementation on the immune response of old people. Am J Med 1981;70:1001–4.

[9] Miller LT, Kerkvliet NI. Effect of vitamin B6 on immunocompetence in the elderly. In: Bendich A, Chandra RK, editors. Micronutrients and immune functions. New York, New York: Academy of Sciences, 1990. p. 49–54.
[10] Penn ND, Purkins L, Kelleher J, et al. The effect of dietary supplementation with vitamins A, C and E on cell-mediated immunity function in elderly long-stay patients. Age Ageing 1991;20:169–74.
[11] Suboticanec K, Stavljenic A, Bilic-Pesic L, et al. Nutritional status, grip strength and immune function in institiutionalised elderly. Int J Vitamin Nutr Res 1989;50:20–8.
[12] Chavance M, Hebeth B, Fornier C, et al. Vitamin status, immunity and infection in an elderly population. Eur J Clin Nutr 1989;43:827–35.
[13] Meydani SN, Meydani M, Blumberg JB, et al. Vitamin E supplementation and in vivo immune response in healthy elderly subjects. JAMA 1997;277:1380–6.
[14] Chandra RK. Graying of the immune system. Can nutrient supplements improve immunity in the elderly? JAMA 1997;277:1398–9.
[15] Chandra RK. Effect of vitamin and trace element supplementation on immune responses and infection in elderly subjects. Lancet 340:1124–7.
[16] Jain AL. Influence of vitamins and trace-elements on the incidence of respiratory infection in the elderly. Nutr Res 2002;22:000–000.