Acute respiratory infections in elderly people: the role of micronutrients and lifestyle

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ABSTRACT

Acute respiratory infections are the most frequent of all infectious diseases. In popular speech common cold, flu (influenza), and pneumonia all denote acute respiratory infections. Elderly people show an increased risk of these infections and their complications. In The Netherlands about 2.000 elderly people die annually from influenza and influenza-related illness. Because the number of elderly people is growing rapidly worldwide, factors that could diminish the risk of the infections, may have great public health importance.

This thesis focuses on the effects of micronutrients and lifestyle on acute respiratory infections in 652 apparently healthy well-nourished elderly people of 60-95 years. Our 15-months randomized controlled trial showed that neither multivitamins-minerals in doses near the recommended dietary allowance nor 200mg of vitamin E had beneficial effects on the incidence and severity of acute respiratory infections. Incidence rate ratio (95% confidence interval) was 0.95 (0.75-1.15) in groups that received multivitamins-minerals and 1.12 (0.88-1.25) in groups that received vitamin E. Instead, we observed unfavorable effects of vitamin E on illness-severity. In the vitamin E compared to the no vitamin E group, illness-duration was 19 versus 14 days (p=0.02); no. of symptoms was six versus four (p=0.03); presence of fever was 37% versus 25% (p=0.009); and presence of activity-restriction was 52% versus 41% (p=0.02). Episodes of respiratory infection were self-assessed by means of a diary and substantiated by nurse telephone check, home-visits, and microbiology and serology testing in a subset. Infections turned out to be laboratory confirmed in 58% of the patients, whereas in only four percent of persons without symptoms of infection a pathogen was identified. Major pathogens in patients suffering from acute respiratory infection were rhinoviruses (32%), coronaviruses (17%), and influenzaviruses (7%).

In addition, observational analyses were performed using the data of the trial. High plasma beta-carotene concentrations may reduce the incidence of acute respiratory infections, whereas alcohol consumption may increase the risk. Observational studies are however sensitive to bias and the influence of plasma carotenoids and lifestyle factors, i.e. alcohol consumption, smoking, and physical activity, on acute respiratory infections remain therefore subject to debate. Our main conclusion is that relatively healthy, well-nourished elderly people will not benefit from multivitamin-mineral and vitamin E supplementation in reducing acute respiratory infections.

CONTENTS

1

General introduction

Acute respiratory infections are a challenge for those interested in improving the health of elderly people. These infections represent over 50% of all types of infectious diseases, followed by genitourinary, skin, and gastrointestinal infections.[1] Acute respiratory infections are often accompanied by complications and exacerbations of chronic obstructive pulmonary disease, which result in a large burden of morbidity and mortality.[2;3] Furthermore, the infections are one of the most common reasons for medical consultation.[4] In The Netherlands, on average yearly 400/10.000 persons contact their general practitioner because of acute respiratory infections, and 2.000 elderly persons die from influenza infection or influenza-like illness.[5-7]

It is worthwhile to try to gain better insight in the role of modifiable nutritional and lifestyle factors influencing acute respiratory infections. Examples of such factors are micronutrient status, alcohol consumption, smoking, and physical activity. If such factors indeed influence acute respiratory infection, their modification may produce great health benefit.

1.1 EPIDEMIOLOGY OF ACUTE RESPIRATORY INFECTIONS

1.1.1 Symptomatology

The symptom patterns evoked by the different respiratory viruses are indistinguishable from each other.[8-10] Commonly occurring symptoms are sneezing, rhinorrhea, cough, sputum production, sore throat, hoarseness, pain when taking a deep breath, shortness of breath, fever, chills, headache, fatigue, lacrimation, and myalgia.[9;11] Acute respiratory infection is therefore a generic term for respiratory illness caused by several pathogens. In popular speech, common cold, flu, and pneumonia all denote acute respiratory infections. Sometimes, a distinction is made between upper and lower respiratory tract infections depending on the

symptomatology.[8;11] In case of self-reporting of symptoms it is hard to make such a distinction, because lower respiratory tract symptoms were reported to complicate 65% of upper respiratory tract infections.[8] In this thesis, the infections are simply referred to as 'acute respiratory infection'.

1.1.2 Diagnosis

Assessment of acute respiratory infections is mostly based on clinical symptoms, such as sneezing, rhinorrhea, cough. In population-based studies, symptoms are often self-reported by the participants in a retrospective questionnaire or a prospective diary. Assessment of symptoms is occasionally performed by weekly or monthly nurse or physician contacts in which symptoms are retrospectively recorded.[8;12-16] Physical examination by a physician or nurse during an episode is a more valid way of diagnosis as compared to self-assessment, although still subjective. Objective ways to validate the diagnosis of symptoms are for example radiography of chest and sinuses, or laboratory tests of blood and sputum analyzing immunological parameters or the presence of causative microorganisms.[17-19] However, these methods are in general invasive and too expensive to be applied in large scale epidemiological studies.

1.1.3 Incidence

Incidence of acute respiratory infections is age specific. Protection from acute respiratory infections is observed during the first six months of life. Maternal antibodies could explain this phenomenon.[20;21] Incidence of respiratory infections was highest in infants six months to one year, i.e. ten infections per infant per year.[21] With increasing age, a gradual acquisition of resistance to respiratory infections was reported. From birth until five years of age, it is estimated that children experience six respiratory infections per year.[21] On average, adults and

elderly persons acquired one to two acute respiratory infections per person per year.[3;8;20] Exception was an increased incidence in young adult women having two to three infections per person per year. This might be explained by a greater exposure to infected young children.[20]

Lower respiratory tract infections had a median duration of 12 days, whereas upper respiratory illness had a median duration of only four days in families with children.[22] In elderly persons, acute respiratory infections had a median duration of 15-16 days.[8;23]

1.1.4 Causative agents

The major part of the acute respiratory infections has a viral origin. In adult patients with medical consultation for respiratory infection, 50% of the infections had a viral, 12% a bacterial, and 20% an atypical origin.[24] Common viruses are rhinovirus, coronavirus, influenzavirus A and B, enterovirus, and respiratory syncytial virus.[8;11;12;20;25;26] A number of new viruses, such as human metapneumovirus, have been recognized in recent years.[27;28] Little is known about the prevalence of respiratory virus infections in elderly persons. Recently, it was reported that rhinovirus infections cause substantial morbidity in elderly persons.[3;8]

1.1.5 Subclinical respiratory infections

Information on subclinical respiratory infections is sparse. Such persons without any symptoms of acute respiratory illness, may act as an unrecognized source of respiratory infections and transmit the pathogen to others. Johnston et al. showed that 12% of the children had subclinical rhinovirus or enterovirus infections, whereas in four percent of the asymptomatic adults a subclinical infection of these viruses was observed.[29] Preliminary results of a Dutch study revealed that subclinical infections occurred in 19% of persons of all

age categories consulting their general practitioner.[5] No data is available on subclinical infections in asymptomatic elderly persons.

1.2 AGEING AND ACUTE RESPIRATORY INFECTIONS

Many risk factors for acute respiratory infection and its complications have been reported, such as age, institutionalization, immunosuppression, chronic obstructive pulmonary disease, asthma, and other comorbid conditions, season of the year, social-economical status, smoking habits, and alcoholism.[20;21;27;30] An age-related decline in immune response is thought to be an important cause of the increased risk as observed in elderly individuals. Impairments of mainly cellular, but also humoral, and innate immunity are observed in the older population.[31] Age-related modifications in the cell-mediated immunity are for example a reduced production of interleukin-2, a decreased number of T-Helper (TH) 1 cells resulting in a disturbed TH1-TH2 cell ratio, and an increased production of prostaglandin E2, which is an inhibitor of lymphocyte proliferation.[32;33] Human TH1 cells develop in response to intracellular bacteria and viruses, and could therefore be important in the defense against many pathogens evoking acute respiratory infections.[34]

Apart from immunosenescense-related changes, a diminished defense of the respiratory tract against inhaled microbes may also explain the increased susceptibility in elderly people. A decreased ciliary beating and ultrastructure may play a crucial role in the diminished mucociliary clearance.[35]

Ageing might thus be seen as a factor influencing the susceptibility to acute respiratory infections. Because the number of elderly people is growing rapidly worldwide, their health

needs are a major concern.[1] It is worthwhile to investigate modifiable factors that could influence the susceptibility to the infections. It is suggested that micronutrients, e.g. antioxidant (pro-) vitamins, and lifestyle factors, such as alcohol consumption, smoking, and physical activity, might influence immune response and infectious diseases. If a change of such factors would indeed result in a diminished incidence and/or severity of acute respiratory infections, this can contribute to 'healthy ageing'. The factors are discussed more in detail below.

1.3 THE ROLE OF MICRONUTRIENTS

1.3.1 Multivitamins-minerals

Micronutrients probably play a major role in the immune response of elderly individuals.[32] Inadequate micronutrient status is reported in both the more frail and vulnerable institutionalized elderly persons, as well as in apparently healthy noninstitutionalized older individuals. However, the prevalence seems to be higher in institutionalized people.[36-39] Micronutrient deficiencies are especially prevalent for the vitamins C, D, and B6.[37;38;40;41]

Even in healthy elderly persons, supplementation with modest amounts of vitamins and minerals resulted in an improved immune response, such as a higher number of certain T-cell subsets, interleukin-2 levels, and antibody response to influenza virus vaccine.[19;32;42] Intervention trials on multivitamin-mineral supplementation in noninstitutionalized elderly people showed a reduced illness-duration, but no effect on the incidence of infectious diseases was reported.[15;19;43]

1.3.2 Antioxidant (pro-) vitamins

The pro-oxidant/antioxidant balance is an important determinant of immune cell function, including maintaining the integrity and functionality of membrane lipids, cellular proteins, and nucleic acids. Furthermore, the balance plays a role in controlling signal transduction and gene expression in immune cells. An age-related increase in free radical formation, resulting in a disturbance of the pro-oxidant/antioxidant balance, is the basis for the free radical theory of ageing. In the context of this theory, antioxidants from the diet can influence the primary 'intrinsic' ageing process as well as several secondary age-associated pathological processes, such as cardiovascular disease, cancer and diabetes.[44-46] Because of the antioxidant capacities of vitamin E and carotenoids, such nutrients could be of importance in the antioxidant defense system of the human body. This may result in an improved immune response and possibly in a diminished risk for acute respiratory infections in elderly people.[47]

1.3.3 Vitamin E

Many researchers have investigated the effect of vitamin E on human immune response. Positive effects were mainly reported at high doses of vitamin E, such as an elevated delayed type hypersensitivity test response, increased interleukin-2 production, reduced prostaglandin E2 synthesis, and increased antibody titer responses to certain vaccines.[13;48] The beneficial effects of high dose vitamin E on immune response might be obtained via a direct effect of vitamin E on T cells, but T cell responsiveness might also be improved by a reduced macrophage prostaglandin E2 production.[46;49] In contrast with the extensively investigated effect of vitamin E on immune parameters, studies on vitamin E and infectious diseases are scarce. One study investigated the effect of 200 and 400mg of vitamin E on the incidence of pulmonary infections in institutionalized elderly persons, showing no effect.[16] Two other intervention trials in noninstitutionalized elderly persons investigated infectious disease

incidence only as a secondary outcome: one showed a 30 percent lower incidence, while the other showed a non-significant higher incidence of upper respiratory tract infections.[13;50]

1.3.4 Carotenoids

Carotenoids are fat-soluble pigments found in fruit and vegetables, giving fruit and vegetables their yellow, orange, red, and green colors. Humans are not able to synthesize carotenoids themselves; fruit and vegetables are the main sources of carotenoids for us.[51] Beta-carotene, alpha-carotene, beta-cryptoxanthin, zeaxanthin, lycopene, and lutein are frequently occurring carotenoids in human blood, together comprising approximately 90% of the total plasma pool of carotenoids.[52;53] Several researchers observed an improved immune function after a diet high in fruit and vegetables. It has been suggested that the antioxidant properties of carotenoids could be a causative factor in this mechanism.[54] Studies on carotenoid supplementation or plasma carotenoid status in relation to human immune response were however less convincing. Part of those studies observed an improved immune function,[55-58] while others did not.[58-63] An improved immune response could result in diminished illness, but research on hard outcomes such as respiratory infections is scarce. So far, only one study investigated the effect of beta-carotene supplementation on common cold incidence, showing no effect.[64]

1.4 THE ROLE OF LIFESTYLE

Lifestyle factors, such as alcohol consumption, smoking, physical activity, eating breakfast, hours of sleep and work, and mental stress, are modifiable factors that are thought to influence immune response and therefore possibly the risk of acute respiratory infections.[65] We focused

on three lifestyle factors, i.e. alcohol consumption, smoking, and physical activity, which are discussed in detail below.

1.4.1 Alcohol consumption

Alcohol consumption is reported to influence the immune system, although it is disputable whether effects are beneficial or unfavorable. The dose and type of alcohol, and whether it is consumed chronic or acute, might play a role in the consequences for the immune system. It is suggested that the effect of alcohol consumption on disease risk could be described by an U- or J-shaped curve, reflecting an increased risk for both heavy consumers and abstainers and a diminished risk for moderate alcohol consumers.[66] No or even unfavorable effects of chronic and acute moderate alcohol consumption on immune response have however also been reported.[67;68] Consumption of wine may have beneficial effects on immune response, possibly because of the antioxidant properties of wine.[69] In contrast, absence of effect of moderate wine consumption on immune response has also been observed.[67] In line with the contradictory results of alcohol consumption on immune response, studies on respiratory infections are inconclusive as well. Both an inverse relation between alcohol consumption and respiratory infections was observed,[70] and the opposite.[71] Others concluded that only wine consumption is inversely associated with the incidence of common cold, whereas other alcoholic beverages are not.[72]

1.4.2 Smoking

Cigarette smoke contains several components that can cause harm, such as nicotine and oxidants.[73] Cigarette smoke also contains inducers of reactive oxygen species that can result in oxidative damage.[74] Increased oxidative damage may have unfavorable effects on the immune system and therefore possibly on acute respiratory infections.[75;76] Pathologic changes

of the respiratory tract, such as loss of cilia have also been reported in both active and passive smokers.[77;78] Smoking is therefore reported to be a strong independent risk factor for pneumonia and other respiratory infections in nonelderly adults.[2;70;79;80] Correspondingly, an increased risk for lower respiratory tract complications during acute respiratory infectious diseases has been reported in smokers.[23]

1.4.3 Physical activity

A commonly observed phenomenon in ageing is a gradual adoption of a more sedentary lifestyle. A sedentary lifestyle may result in weight gain and increased risk of chronic and acute illness.[81] Immediately after moderate physical activity and in people with moderate physical activity training an improved immune function was observed, e.g. natural killer cells increased in number and function.[82] The intensity of physical activity is thought to play a role in its effect on immune response. Moderate physical activity might stimulate the immune response, but exhausting physical activity, such as in athletes, could have a suppressant effect.[83-85] Sports and leisure time activities contribute to physical activity, although in elderly people household activity is the most important component.[86] So far, physical activity studies mainly addressed sports activities. Concerning respiratory infections, an increased resistance to or a diminished symptomatology of respiratory infections in people with moderate exercise training was observed by some researchers, while others did not.[87-89]

1.5 RATIONALE AND OUTLINE OF THESIS

As the proportion of elderly people is dramatically increasing and especially elderly persons show an increased susceptibility to the frequent acute respiratory infections and their

complications, it is of great interest to determine whether nutritional and lifestyle factors have an effect on the infections. Our main aim was to investigate the effect of long-term multivitamin-mineral and vitamin E supplementation on the incidence and severity of acute respiratory infections in a large group of elderly persons. The other components of this thesis are observational analyses based on information from this intervention trial.

First, we wanted to have insight in the causative viruses of acute respiratory infections in elderly people and to determine the prevalence of subclinical viral infections in this population. Besides, the results of this study were used to validate the self-report of acute respiratory infections as described in Chapter 3 and 5. Therefore, we investigated the following research question: **What is the percentage positive testing of the most common respiratory viruses, including *Mycoplasma pneumoniae*, in elderly people both with and without symptoms of acute respiratory infection**. A prospective observational study was conducted in a subpopulation of the participants we recruited for our intervention trial, to investigate this research question (**Chapter 2**).

As discussed in the preceding pages, multivitamins-minerals in doses near the recommended dietary allowance (RDA) and high doses of vitamin E are reported to be beneficial in improving immune response and therefore possibly in reducing the risk of acute respiratory infections. We investigated the following research question: **What is the effect of multivitamins and minerals in doses near the RDA and of vitamin E (200mg) on the incidence and severity of acute respiratory infections in elderly people**. We conducted a randomized, placebo-controlled double-blind intervention trial of nutritional supplementation on respiratory infections in a large group of elderly individuals to investigate this research question. This trial is described in **Chapter 3**.

Persons with a high plasma carotenoid status are supposed to have an improved immune response. This could result in diminished risk of acute respiratory infections. However, the relation with respiratory illness has only been investigated for beta-carotene in adult male smokers. Other main carotenoids found in human blood - alpha-carotene, beta-cryptoxanthin, lycopene, lutein, and zeaxanthin - have never been investigated in relation to respiratory infections. Therefore, we investigated the following research question: **What is the relation between the plasma status of six major carotenoids and the incidence and severity of acute respiratory infections in elderly persons**. As described in **Chapter 4**, a retrospective analysis was conducted to investigate this research question.

Apart from dietary factors as described above, lifestyle factors such as alcohol consumption, smoking, and physical activity, could also influence immune response and the susceptibility to infectious diseases. Associations are not well defined: moderate physical activity may have a beneficial effect, smoking can have an unfavorable effect, and the influence of alcohol consumption on acute respiratory infections is still under debate. Associations have never been investigated in elderly people. Therefore, we examined the following research question: **What is the relation between alcohol consumption, smoking, and physical activity and the incidence and severity of acute respiratory infections in elderly persons**. **Chapter 5** describes a prospective observational analysis investigating the relation between the lifestyle factors and acute respiratory infections in the elderly people recruited for the intervention trial.

Finally, the main results, critical design issues, conclusions, public health implications, and suggestions for future research are discussed in **Chapter 6**.

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2

A prospective community-based study on virological assessment among elderly people with and without symptoms of acute respiratory infection

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ABSTRACT

Community-based studies in elderly people concerning microbiology of acute respiratory infections are scarce. Data on subclinical infections are even totally absent, although asymptomatic persons might act as a source of respiratory infections. In an one-year community-based study, we prospectively investigated the possible virological cause of acute respiratory infections in 107 symptomatic case-episodes and 91 symptom-free control-periods. Participants, persons ≥60 years, reported daily the presence of respiratory symptoms in a diary. Virological assessment was performed by Polymerase Chain Reaction (PCR) and serology.

In 58% of the case-episodes a pathogen was demonstrated, the most common being rhinoviruses (32%), coronaviruses (17%) and influenzaviruses (7%). The odds ratio for demonstrating a virus in cases with symptoms versus controls without symptoms was 30.0 (95% confidence interval 10.2-87.6). In four percent of the symptom-free control-periods a virus was detected.

This study supports the importance of rhinovirus infections in community-dwelling elderly persons, whereas asymptomatic elderly persons can also harbour pathogens as detected by PCR and thus might be a source of infection for their environment.

2.1 INTRODUCTION

Elderly people have an increased susceptibility for respiratory infections and related complications.[1] On average, community-dwelling elderly people suffer from one to two acute respiratory infections per year.[2,3] Medical consultation and hospitalization because of such an infection has been reported in 40% and 0.8% of community-dwelling elderly people respectively, during the winters of 1992-3 and 1993-4 in England.[2]

Viruses play a crucial role in acute upper respiratory tract infections, the most common being rhinoviruses, coronaviruses, influenzaviruses and respiratory syncytial viruses.[4] However, laboratory diagnosis of acute respiratory infections in symptomatic elderly people so far focused on institutionalized elderly persons,[5-7] on patients reporting for medical consultation,[8,9] and to a far less degree on community-dwelling elderly persons.[2] Besides, no data are available on the presence of respiratory pathogens in asymptomatic elderly persons. Asymptomatic people with a subclinical infection might however transmit the pathogen to other persons and act as an unrecognized source of respiratory infections.

Therefore, in this prospective community-based study, we investigated the presence of known respiratory viruses in elderly persons both with and without symptoms of an acute upper respiratory tract infection. Second, we compared the clinical characteristics of persons suffering from an acute respiratory infection, during episodes with positive and negative virological laboratory diagnosis.

2.2 METHODS

2.2.1 Subjects

Persons with and without symptoms of an acute respiratory infection, hereafter referred to as cases and controls, were recruited from October 1, 1998 until October 1, 1999 from an intervention trial investigating the effect of micronutrient supplementation on acute respiratory infections in community-dwelling elderly persons (≥60 years).[3] During the one-year study period, a diary was used daily by all participants for reporting symptoms that indicated an acute respiratory infection. Participants were requested to report the onset of symptoms of a possible infection to the study nurse. A subject was identified as case if (1) he/she had respiratory symptoms with a sudden onset; (2) rhinorrhoea/sneezing, sore throat/hoarseness or dry cough were present for at least two days; and (3) the symptoms had a pattern that differed from any usual symptoms.[10,11] Apart from a check by telephone, the study nurse evaluated the symptoms of cases during home visits. From those cases that reported their symptoms within three days to the study nurse every other case, with a maximum of five cases per week, was selected for virological assessment. Cases who reported their symptoms after three days to the study nurse were excluded for virological assessment to overcome false negative test results. Each case-episode, i.e. the period during which a case had respiratory symptoms, had to have been preceded by a seven days symptom-free period. During the one-year study period, 624 incident case-episodes were reported by 346 cases. In total 107 (17%) case-episodes - reported by 97 cases - were selected for virological assessment.

For each case-episode an asymptomatic control was selected as follows. Participant numbers, including all cases and controls, ranged from 1-652. If the participant number of the case was 325 or lower a closest eligible control was selected on participant number by counting back on these numbers. If the participant number of the case was 326 or higher, a closest eligible

control was selected by counting forward on these numbers. Controls were subjects without symptoms of a respiratory infection within a time window of eight weeks before and eight weeks after the symptomatic period of the index case. The study nurse checked the absence of symptoms at the time of recruitment of the control and the diary was checked for absence of symptoms in the previous eight weeks. In total 99 controls were selected.

Cases and controls were matched on age (plus or minus five years) and sex and they were not living in the same house or apartment, did not have chronic obstructive pulmonary disease, asthma or cancer, and did not use severe immunosuppressive medication.

Afterwards, we excluded eight of the 99 originally enrolled controls because they developed symptoms after a median duration of 21 days (range 9-54). For six excluded controls serologic testing was negative, while for two it was missing. With Polymerase Chain Reaction (PCR) two times a rhinovirus and two times a coronavirus OC43 was detected in the eight excluded controls. Results presented are therefore based on the 107 case-episodes and 91 control-periods.

This study was approved by the Medical Ethics Committee of the Wageningen University, The Netherlands and written informed consent was obtained from all participants prior to the study.

2.2.2 Data collection

All participants filled out a questionnaire concerning relevant subject characteristics at baseline. A diary was used daily for self-report of symptoms that indicated an acute respiratory infection. Apart from the symptoms that had to be present because of our case-definition (rhinorrhoea/sneezing, sore throat/hoarseness, dry cough), also accompanying symptoms were recorded in the diary: (1) symptoms of a lower respiratory tract infection (sputum production, wheezing, pain on respiration); (2) systemic symptoms (fever (self-

assessed by a supplied thermometer), malaise, headache, rigors, muscular pain, perspiration); (3) other symptoms (tearful eyes, pain in facial sinuses or ear); (4) restriction of activity (staying in bed, not being able to do daily activities, staying at home); (5) episode-related medication, including antibiotic use; (6) medical consultation; and (7) hospitalization.[2] If the study nurse judged during a home-visit the case's symptoms as an acute respiratory infection, in both the case and the matched control an acute phase serum sample and one swab from the nose and one from the throat were taken within three days and a convalescent serum sample was taken within two to four weeks after onset of the first symptoms of the case. Samples in cases and controls were taken on the same day to exclude seasonal differences.

2.2.3 Microbiological diagnosis

PCR or serology was used to diagnose infection with the eight most common respiratory viruses and *Mycoplasma pneumoniae* (*M. pneumoniae*). PCR was performed for those viruses for which either no or only aspecific serology was available and for which validated PCR tests were available in our lab. Infections with rhinovirus, enterovirus, coronavirus OC43 and 229E, and respiratory syncytial virus were diagnosed by PCR. Serology was performed for those viruses for which either no PCR was available, or the nucleic acid extraction method had to be changed for DNA isolation (in the case of *M. pneumoniae*). Infections with influenzavirus A and B, parainfluenzavirus 1, 2 and 3, adenovirus and *M. pneumoniae* were diagnosed by serology.

Polymerase chain reaction

Swabs from the nose and from the throat, hereafter referred to as 'nose/throat samples', were placed together in four milliliters Hanks' balanced salt solution containing gelatin, lactalbumin, yeast, and antibiotics. Upon receipt of the nose/throat samples at the laboratory,

the swabs were twirled in the transport medium and removed. An aliquot of 200µl of the sample was used for nucleic acid extraction by using the High Pure RNA isolation kit (Boehringer, Mannheim, Germany). Five microliters of the eluted RNA preparation was used in a 25µl single-tube RT-PCR followed by a nested-PCR using primer pairs as described previously for rhinovirus and enterovirus.[12] Another five microliters of extracted RNA was used in a single 25µl single-tube reverse transcriptase-polymerase chain reaction (RT-PCR) followed by a nested-PCR using primer pairs as described previously for respiratory syncytial virus (RSV) and coronavirus OC43 and 229E in a multiplex format.[13,14]

In the RNA isolation procedure and PCR-method for RSV detection, sensitivity for RSV-A was about one virus particle and for RSV-B about 70 virus particles. The virus particle count was determined by quantitative EM (Advanced Biotechnologies Incorporated, Columbia, Md). Positive controls from culture were used in each PCR-test for the respective viruses. To prevent carry-over contamination within the laboratory, preparation of the patient samples and PCR mixtures was performed in safety hoods in separate dedicated positive pressure laboratories. To check for carryover contamination of samples and for amplicon contamination during the procedure, negative controls, consisting of transport medium, were included after every fifth patient sample. Subjects with a positive PCR result were considered to be infected by a known virus, which was interpreted as a laboratory-confirmed infection.

Serology

Paired sera from all cases and controls were analyzed for immunoglobulin (Ig) G antibodies against influenzavirus A and B, adenovirus, and *M. pneumoniae.* For parainfluenzavirus 1, 2 and 3, IgA antibodies, combining the three antigens in one assay, were detected. Analyses were performed using commercially available ELISA (Serion Immunodiagnostica GmbH, Würzburg, Germany), and quantitative results, expressed as units/milliliter, were calculated

using a lot specific standard curve and calculation table as supplied in the test kit. Results were interpreted as negative, indeterminate or positive according to the manufacturer instructions. In the case of indeterminate results for the parainfluenza IgA assay on paired sera, detection of total antibodies against separate parainfluenza 1, 2, and 3 antigens was repeated in a complement fixation assay (CFA), using commercially available parainfluenza 1, 2, and 3 antigens (Virion, Ruschlikon, Switzerland). In Elisa's a change from negative to positive result, and in the CFA a fourfold rise in antibody titer between the paired sera, were interpreted to be a laboratory-confirmed respiratory infection.

2.2.4 Statistical analyses

Data analysis concerning virological (including *M. pneumoniae*) assessment was performed with the 107 case-episodes and the 91 control-periods. Differences in the distributions for continuous data, i.e. age, self-perceived health, and illness-duration were compared with Independent Sample Student's T-test. Illness-duration was not normally distributed and was log transformed to obtain normality. A Chi-Square test or a Fisher's Exact Test was used to test the correlation between discrete variables, i.e. sex, influenza vaccination, smoking habits, allergy, sharing an apartment, presence of microorganisms, symptoms of a lower respiratory tract infection, systemic and other symptoms, restriction of activity, fever, medical consultation, hospitalization, episode-related medication and episode-related antibiotic use. A Fisher's Exact Test was used to calculate the odds ratio for demonstrating a virus in cases with symptoms of acute respiratory infection versus controls without symptoms of such an infection. Alpha was taken as 0.05 in all analyses.

2.3 RESULTS

The matching-procedure on sex and age resulted in well-balanced groups of cases and controls with respect to these and other relevant variables (Table 2.1). Micronutrient supplementation related to the intervention trial was also similar between cases and controls.[3]

Table 2.1 Characteristics of persons with (cases) and without (controls) symptoms of an acute respiratory infection, in whom virological (including *M. pneumoniae*) tests were performed

	Cases (n=97)	Controls (n=91)
Age (years), mean (SD)	72.2 (6.8)	72.2 (5.6)
Men	44 (45%)	47 (52%)
Self-perceived health (range 1-10), mean (SD)	7.5 (1.2)	7.5 (1.2)
Influenza vaccination in 1998	73 (75%)	73 (80%)
Current smoker	50 (52%)	45 (49%)
Former smoker	7 (7%)	3 (3%)
Allergy*	12 (12%)	11 (12%)
Sharing an apartment	61 (63%)	64 (70%)

Data are n (%) unless otherwise indicated.

*Allergy against house-dust mite and feces, pollen grains, domestic pets or moulds.

The 97 symptomatic cases had 107 case-episodes of respiratory infection during which virological (including *M. pneumoniae*) tests were performed. In 62 (58%) of these case-episodes at least one microorganism was demonstrated, whereas in two of these 62 two different microorganisms were demonstrated. In 45 (42%) case-episodes none of the applied

Table 2.2 Viruses (including *M. pneumoniae*) demonstrated in symptomatic case-episodes of acute respiratory infection and symptom-free control-periods of community-dwelling elderly persons, in The Netherlands from October 1, 1998 until October 1, 1999

	Case-episodes (n=107)	Control-periods (n=91)
Negative microbiology	45 (42%)*	87 (96%)
Rhinoviruses†	34 (32%)	2 (2%)
Coronavirus (OC43+229E)†	18 (17%)	2 (2%)
Influenzavirus A	5 (5%)	0
Influenzavirus B†	2 (2%)	0
Enterovirus	2 (2%)	0
Parainfluenzavirus (1, 2 + 3)	2 (2%)	0
Mycoplasma pneumonia	1 (1%)	0
Respiratory syncytial virus	0	0
Adenovirus	0	0

*Significantly different with symptom-free controls, p<0.0001.
†During two case-episodes two viruses were demonstrated: one case-episode with rhinovirus + coronavirus OC43 and one with coronavirus OC43 + influenzavirus B.

tests was positive. Of ten cases two case-episodes were included. For seven out of the mentioned ten cases, test results were different, i.e. different pathogens, or negative in one and positive virology in the other episode. In two cases both episodes had negative virology. Only in one case, in both episodes rhinovirus was detected. The most common viruses demonstrated were rhinoviruses (32%) and coronaviruses (17%) followed by influenzaviruses (7%), enteroviruses (2%), parainfluenzaviruses (2%), and *M. pneumoniae* (1%). Respiratory syncytial virus and adenovirus were not detected. Three of the seven cases diagnosed with an influenzavirus infection had been vaccinated against influenza. None of the titer rises on

which the influenzavirus infection was diagnosed, was related to vaccination, as two to four months passed between vaccination and the diagnosis of an influenzavirus infection. Presence of rhinovirus infections was almost five times higher compared to influenzavirus infections in this community-dwelling elderly population (Table 2.2).

Table 2.3 Relation between virology and clinical characteristics in community-dwelling elderly persons during symptomatic episodes of acute respiratory infection

	Rhinoviruses (n=34)	Coronavirus OC43+229E (n=18)	Influenzavirus A and B (n=7)	Negative virology (n=45)	Case-episodes total (n=107)
Episode duration (days), median (min, max)	9 (2, 41)	7 (2, 34)	12 (9, 32)††	8 (2, 29)	9 (2, 41)
Symptoms of lower respiratory tract infection*	20 (58.8%)	9 (50.0%)	5 (71.4%)	21 (46.7%)	57 (53.3%)
Systemic symptoms†	19 (55.9%)	12 (66.7%)	7 (100.0%)††	30 (66.7%)	68 (63.6%)
Other symptoms‡	15 (44.1%)	6 (33.3%)	3 (42.9%)	14 (31.1%)	39 (36.4%)
Restriction of activity§	11 (32.4%)	6 (33.3%)	6 (85.7%)	14 (31.1%)	39 (36.4%)
Fever	3 (8.8%)††	5 (27.8%)	5 (71.4%)‡‡	10 (22.2%)	25 (23.4%)
Medical consultation**	3 (8.8%)	2 (12.5%)	4 (57.1%)	4 (9.1%)	14 (13.5%)
Hospitalization**	0	0	0	0	0
Medication**	12 (35.3%)	6 (37.5%)	3 (42.9%)	22 (50.0%)	45 (43.3%)
Antibiotic use**	2 (5.9%)	0 (0.0%)	2 (33.3%)	2 (4.6%)	7 (6.8%)

Data are number of case-episodes (%) unless otherwise indicated.

*Lower respiratory tract symptoms: sputum production, wheezing, and pain on respiration.

†Systemic symptoms: malaise, headache, rigors, muscular pain, and perspiration.

‡Other symptoms: tearful eyes, pain in facial sinuses, pain in ear.

§Restriction of activity: staying in bed, staying at home, not able to do daily activities.

**Data of three case-episodes are missing.

††$p < 0.05$ compared with case-episodes total.

‡‡$p < 0.01$ compared with case-episodes total.

In four out of 91 control-periods (4%) a virus was demonstrated, i.e. two times a rhinovirus and two times a coronavirus. Two out of these four controls with positive virology never showed symptoms of a respiratory infection during the one-year study period. The two remaining controls with positive virology did not have any symptoms at least three and a half and four months before and eight and four months after sample collection, respectively.

Overall, the odds ratio for demonstrating a virus (or *M. pneumoniae*) in cases with symptoms versus controls without symptoms of acute respiratory infection was 30.0 (95% confidence interval 10.2-87.6). Despite small numbers (n=5) significantly more influenzavirus A infections were identified during symptomatic periods in winter (October-March) compared to summer (p=0.02). Enteroviruses, parainfluenzaviruses and *M. pneumoniae* were only detected in summer (April-September).

Clinical characteristics of the persons suffering from an acute respiratory infection, during episodes with positive and negative virological laboratory diagnosis, are described in Table 2.3. Influenzavirus infection was associated with significantly longer illness-duration and more systemic symptoms than the other infections with positive and negative virology. Restriction of activity, presence of fever, medical consultation, and antibiotic use were also more frequently reported during influenzavirus infections, although not significantly different from the other infections with positive and negative virology.

2.4 DISCUSSION

This study shows that subclinical respiratory infections occur in a minor part (four percent) of asymptomatic elderly persons. Besides, we showed the importance of rhinovirus infections in community-dwelling elderly people because of its high frequency.

To our knowledge, this is the first study to investigate several common respiratory pathogens in community-dwelling elderly persons both with and without symptoms of an acute respiratory infection. So far, only two studies reported on microbiological evidence of respiratory infection in community-dwelling healthy subjects with and without symptoms of such an infection. One study focused on detection of rhinoviruses and enteroviruses by PCR in children and adults.[15] In 12% and four percent of the asymptomatic children and adults, respectively, virological assessment was positive. Although Johnston et al.[15] tested only for rhinoviruses and enteroviruses, the frequency of subclinical respiratory infections in those healthy adults is similar to what we observed in our older population. Preliminary results of a Dutch study being performed in persons consulting their general practitioner for signs and symptoms of an acute respiratory infection, showed a positive virological assessment in 19% of the controls.[16] This percentage is higher than observed in our study. However, that study population existed of participants from all age categories, including babies and children. As showed by Johnston et al.[15] the percentage of asymptomatic persons with positive virological assessment is clearly higher in children, which might explain the discrepancy.

Common viral pathogens demonstrated during symptomatic periods in children and adults,[4-17] in institutionalized elderly patients,[7-11] in patients with medical consultation,[8] and in community-dwelling elderly persons[2] are rhinoviruses, coronaviruses, influenzavirus A and B, and respiratory syncytial virus (RSV), which is in line with our results. The frequency of the most common viruses varies between the different subpopulations. Corresponding to one previously performed community-based study in elderly people, we also showed that rhinovirus infections are highly prevalent and can cause a great overall disease burden in this population.[2] Corresponding to the results of Nicholson et al. in community-dwelling elderly persons,[2] but in contrast to studies in more frail elderly persons as those living institutionalized and to studies with a general practitioner-based setting,[7,11,18] we also observed that

influenzavirus infections and RSV infections seem to occur less frequent in free-living elderly people. A severe morbidity is caused by viruses such as influenzavirus and RSV,[19] which is corresponding to our results on influenzavirus infections. This might explain the higher frequency of RSV and influenzavirus infections demonstrated in studies with general practitioner-based or institutionalized settings.[19] In total, four out of seven patients with influenzavirus infection, were not vaccinated against influenza. This might indicate the need for preventive vaccination in elderly persons. In agreement with other studies in institutionalized and in community-dwelling elderly subjects,[2,7,11] we found that infections with parainfluenzavirus, enterovirus, adenovirus, and *M. pneumoniae* are of minor importance in causing acute respiratory infections in elderly persons.

We obtained a microbiological diagnosis in 58% of the case-episodes. The diagnostic deficit of 42% is relatively low, as in most studies a microorganism was demonstrated in at maximum 50% of the case-episodes.[2,7,8] Other, partly new or unknown viruses, bacteria, and atypical microorganisms other than *M. pneumoniae* may be responsible for some of the clinical and possible additional subclinical infections with negative microbiology. Bacterial, atypical, and viral microorganisms in adult patients consulting for respiratory infection have been shown in 12%, 20% and 50% of the patients, respectively.[20] Also *Chlamydia* species are reported to cause acute respiratory infections in community-dwelling elderly persons,[2] although the proportion of bacterial infections is reported to be rare in adult patients with common cold.[4] Besides, *Chlamydia* infections occurred in only one percent of the community-dwelling elderly people, and were mainly analyzed in patients with COPD and asthma, while we excluded those patients.[2,21] However, we cannot exclude that part of the diagnostic deficit in our study might be explained by such bacterial and atypical microorganisms.

Little is known about the time-period after infection during which PCR-based tests are positive.[12,14,22] This issue is especially crucial in interpreting PCR positive results in nose/throat samples obtained from subjects both with and without symptoms of a respiratory infection. Andeweg et al.[12] demonstrated that rhinoviruses were no longer detected by PCR in patients who had recovered from disease. In our study, all cases and controls were followed day-to-day by using a self-reporting diary system. It was therefore possible to include only controls not having any symptoms two months before and two months after sampling. Thus, it is very unlikely that the controls, in which a virus was detected, were in the post-infectious or incubation period of a symptomatic infection. Moreover, in the nose/throat samples of four of the eight excluded controls a respiratory virus was detected and those controls apparently were in the incubation period. Detection of rhinovirus, enterovirus, RSV, and coronavirus infections by the PCR-method has been used before and is widely accepted.[12-14] Although PCR-based tests are highly sensitive and specific, false positives due to contamination of negative samples with PCR product in the laboratory might have occurred.[23] However, given the strict conditions under which PCR was performed,[24] this is not likely. Negative controls included after each fifth test sample were PCR-negative in all samples, indicating that contamination was effectively prevented.

Underreport could have occurred if cases were admitted to the hospital when having an acute respiratory infection. Since none of the cases reported that they have been admitted to a hospital because of an acute respiratory infection or its complications, underreport because of hospitalization is no issue in this study.

The subjects who participated in this study were recruited from an intervention trial studying the effect of micronutrient supplementation on acute respiratory infections. Random selection of participants of this double blind intervention trial resulted in a similar distribution of supplementation between cases and controls. There was no significant correlation between

positive microbiologic testing and (type of) supplementation.[3] Therefore, confounding by the supplementation is likely to be negligible in this study.

In conclusion, rhinovirus infections cause substantial morbidity among community-dwelling elderly persons because of its high prevalence in this population. Also, although definitely more respiratory microorganisms were demonstrated among persons with symptoms of an acute respiratory infection, asymptomatic elderly persons can also harbour respiratory pathogens and thus might be a source of infection for their environment.

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3

Effect of daily vitamin E and multivitamin-mineral supplementation on acute respiratory infections in elderly persons: a randomized controlled trial

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ABSTRACT

Immune response in elderly individuals has been reported to improve after micronutrient supplementation. However, efficacy trials evaluating infectious diseases as outcomes are scarce and inconclusive. Therefore, we investigated the effect of daily multivitamin-mineral and vitamin E supplementation on incidence and severity of acute respiratory infections in elderly individuals in a randomized, double blind, placebo-controlled, 2*2 factorial trial. A total of 652 noninstitutionalized individuals (≥60 years) were enrolled from two community-based sampling strategies in the Wageningen area of The Netherlands, conducted from 1998-2000. At baseline, six percent of the participants had suboptimal ascorbic acid and 1.3% had suboptimal alpha-tocopherol plasma concentrations. Subjects were assigned to physiological doses of multivitamins-minerals, 200mg of vitamin E, both or placebo. Incidence and severity of self-reported acute respiratory infections at 15 months were assessed by nurse (telephone contact), home visits, and microbiological and serological testing in subsets of patients. During a median observation period of 441 days, 443/652 (68%) participants recorded 1024 respiratory infection episodes. The incidence rate ratio of acute respiratory infection for multivitamin-mineral supplementation was 0.95 (95% Confidence Interval 0.75-1.15; p=0.58) and for vitamin E supplementation 1.12 (95% CI 0.88-1.25; p=0.21). Severity of infections was not influenced by multivitamins-minerals. For vitamin E versus no vitamin E, severity was worse: median (interquartile range) for illness-duration was 19 (9-37) vs 14 (6-29) days, p=0.02; number of symptoms 6 (3-8) vs 4 (3-8), p=0.03; presence of fever 36.7% vs 25.2%, p=0.009; and activity restriction 52.3% vs 41.1%, p=0.02. Thus, neither daily multivitamin-mineral supplementation at physiological doses nor 200mg of vitamin E showed a favorable effect on the incidence and severity of acute respiratory infections in well-nourished elderly individuals. Instead we observed adverse effects of vitamin E on illness-severity.

3.1 INTRODUCTION

An age-related decline in immune response may increase the risk of infectious diseases and their complications.[1] Beneficial effects of micronutrient supplementation on immune response have been observed not only in institutionalized older persons but also in healthy noninstitutionalized elderly individuals.[2] Supplementation with multivitamins-minerals at the recommended dietary allowance (RDA) level mainly improved cellular immune parameters.[2;3] For vitamin E, a higher dose, such as 200mg/day, has been required to demonstrate an effect.[4] For infectious disease occurrence, however, evidence demonstrating the efficacy of multivitamin-mineral supplementation at RDA level[3;5] or at a high level[4] is limited and studies have shown contradictory results. More specifically, the effect of multivitamin-mineral supplementation at physiological doses or of 200mg of vitamin E on respiratory infections has not been investigated in noninstitutionalized elderly persons. Results in institutionalized elderly individuals have been inconsistent.[6-8] The aim of our randomized intervention trial was to determine whether long-term daily supplementation with multivitamins and minerals at the RDA level or with 200mg of vitamin E reduced the incidence and severity of acute respiratory infections in noninstitutionalized elderly persons.

3.2 METHODS

3.2.1 Subjects

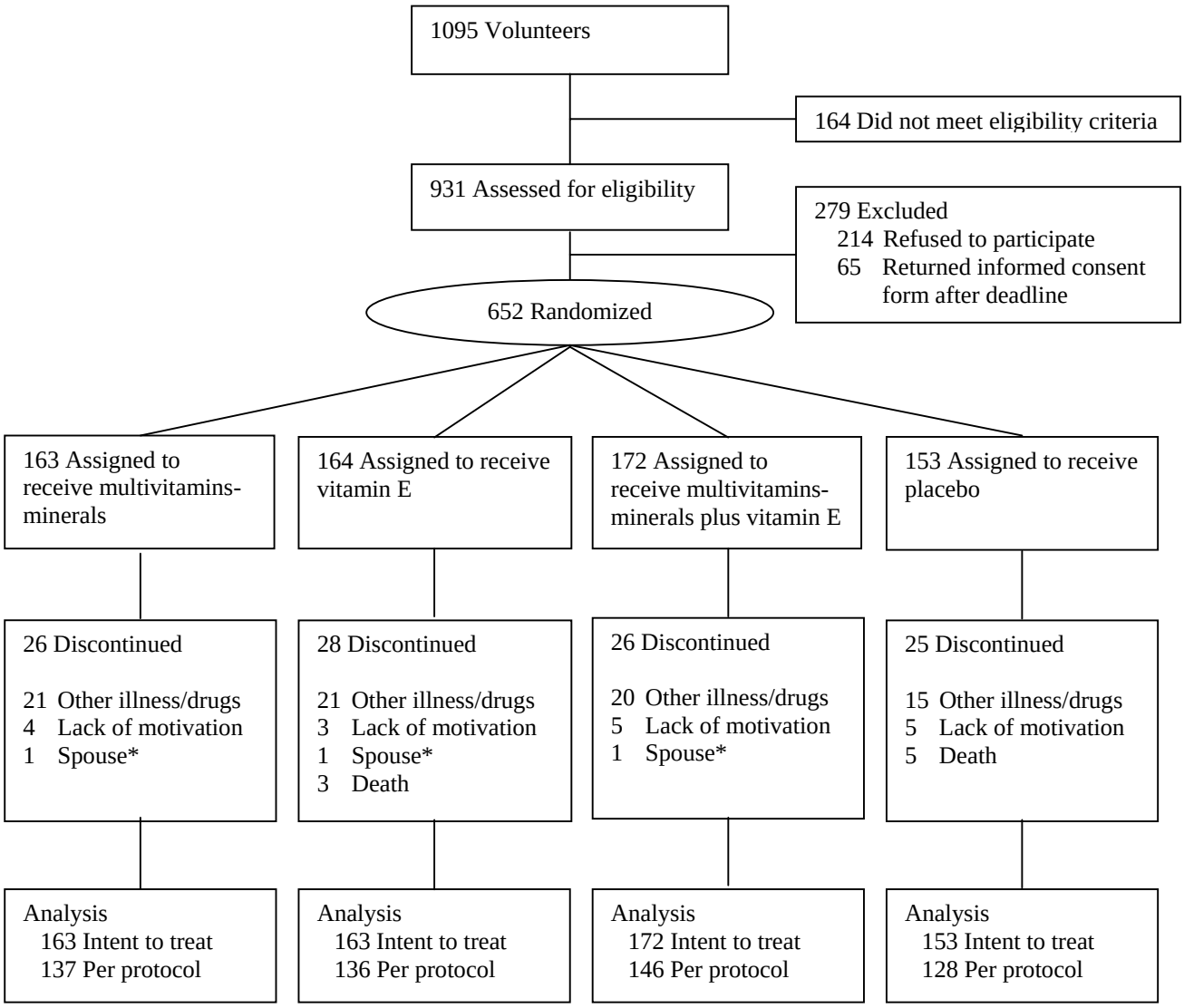
In total 652 men and women (≥60 years) were enrolled in the randomized, double blind, placebo controlled trial. All together, 11.417 individuals were invited to participate through two community-based sampling strategies: via the population registry of the town of

Doetinchem, The Netherlands, and via direct mail to senior citizens' apartments in several other towns. Figure 3.1 shows the flow diagram of participants. Individuals were excluded if they used immunosuppressive treatment, anticoagulants interfering with vitamin K metabolism,[9] dietary supplements in the previous two months, or if they had a history of cancer, liver disease or fat malabsorption during the five years before randomization. Participants were enrolled between September 1, 1998, and March 23, 1999, and were followed up for a maximum of 15 months. Eleven participants in the multivitamin-mineral, ten in the vitamin E, eleven in the multivitamin-mineral plus vitamin E, and six in the placebo groups did not meet the compliance criteria of taking at least 80% of their capsules but were included in both analyses; 26, 28, 26, and 25, respectively were excluded from the per protocol analysis for discontinuing the intervention. None of the reasons for discontinuing intervention were considered a result of the treatment. Written informed consent was obtained prior to study participation. The Medical Ethics Committee of Wageningen University, The Netherlands, approved the research protocol.

3.2.2 Treatment

The following treatments were applied in a 2*2 factorial design: multivitamins and minerals, vitamin E, multivitamins and minerals plus vitamin E, and placebo. The multivitamin-mineral capsule contained: retinol (600µg), beta-carotene (1.2mg), ascorbic acid (60mg), vitamin E (10mg), cholecalciferol (5µg), vitamin K (30µg), thiamin mononitrate (1.4mg), riboflavin (1.6mg), niacin (18mg), pantothenic acid (6mg), pyridoxine (2.0mg), biotin (150µg), folic acid (200µg), cyanocobalamin (1µg), zinc (10mg), selenium (25µg), iron (4.0mg), magnesium (30mg), copper (1.0mg), iodine (100µg), calcium (74mg), phosphor (49mg), manganese (1.0mg), chromium (25µg), molybdenum (25µg), and silicium (2µg).

Figure 3.1 Study flowchart



*Spouse: participant discontinued because of illness or death of spouse.

We chose dosages at RDA levels for vitamins and 25% to 50% of RDA levels for minerals because multivitamin-mineral supplementation near RDA levels previously was shown to decrease duration of infections in noninstitutionalized elderly persons.[3] The vitamin E capsule contained 200mg of dl-alpha-tocopheryl acetate because this dose was suggested to be optimal for improving immune response.[4] Placebo capsules contained soybean oil. Quality

control of the capsules after treatment showed no decrease in the original contents. Each participant received two capsules per day to be ingested with dinner for a maximum of 15 months. A computer-generated, four-per-block, randomization list was created by the pharmacy (Roche Vitamins Europe, Basel, Switzerland), allocating treatment to participant number. Block-randomization was used to obtain balanced groups over seasons. Numbered boxes containing identical-looking capsules were transported from the pharmacy to the Wageningen University. At enrollment, boxes were assigned consecutively to participants. Treatment allocation was kept at the pharmacy exclusively in sealed opaque envelopes, while participant identity was known exclusively at the Wageningen University. None of the treatment codes was broken during the study period. After the investigator performed all analyses, the pharmacy disclosed the treatment list to the Wageningen University. Roche Vitamins Europe provided the randomization code and the supplements, and performed the vitamin concentration analysis in this trial. The company was not involved in the design and conduct of the study, the collection, statistical analyses, and interpretation of the data, or the preparation, review, approval, or control of the manuscript.

3.2.3 Data collection

Participants completed a questionnaire about relevant population characteristics and supplement use at baseline, and yearly influenza vaccination after treatment. Body-mass index was calculated by dividing weight in kilograms by the square of height in meters. Baseline plasma samples were collected for determination of the concentration of alpha-tocopherol, ascorbic acid, retinol, and carotenoids. To monitor compliance, these assessments were repeated in a postintervention sample of a subset (n=300). Returned capsules were counted for all participants. The before and after intervention blood samples were collected between 8.30 and 11.00 AM. A light breakfast, without fruit or fruit juices, was allowed before sampling.

Plasma was stored at –80°C within six hours of blood draw. The reversed-phase high-pressure liquid chromatography method was used to analyze fat-soluble vitamin concentrations.[10-11] Ascorbic acid concentration was obtained via standard procedures and assessed by fluorimetric assay.[12] Total cholesterol levels were analyzed using enzymatic Chod-Pap method with Cobas-Bio centrifugal analyzer.[13] Detection levels and intraday and interday repeatability were within normal ranges and were sufficient for all analyses.[10;11] Suboptimal plasma vitamin levels were based on other studies in elderly persons.[14;15]

3.2.4 Assessment of respiratory symptoms

Main outcomes were incidence and severity of acute respiratory infections assessed using a diary in which participants, who received thorough instruction, recorded all acute symptoms. Acute symptoms were defined as follows (1) sudden onset; (2) a pattern that differed from any usual symptoms; and (3) one or more of the respiratory symptoms like rhinitis, sore throat or cough persisted for at least two days.[16] Presence of accompanying signs was also recorded in the diary: fever, phlegm production, wheezing, pain during breathing, headache, shivering, perspiration, muscular pain, malaise, tearful eyes, pain in facial sinuses, ear pain, staying in bed, restriction of daily activities, staying at home, and use of medication or other treatment. Indicators of severity were defined beforehand as (1) total duration of respiratory episodes; (2) number of symptoms; (3) percentage of participants with fever; (4) restriction of activity; or (5) episode-related medication.

3.2.5 Diagnosis of respiratory infections

Participants were requested to report symptoms of a possible acute respiratory infection by telephone to the study nurse who checked whether the symptoms met the definition. Rectal temperature was self-assessed with a study-supplied thermometer on all symptomatic days.

As a more specific diagnostic test, microbiology by polymerase chain reaction and serology tests were performed in a random subsample of 97 symptomatic patients during 107 illness episodes from October 1, 1998, until October 1, 1999. A nose-throat swab and acute-phase blood sample were taken within three days and a convalescent blood sample was taken between two to four weeks after onset of symptoms. The nose-throat swab and paired blood sample were tested for the nine most common respiratory pathogens: rhinovirus, enterovirus, coronavirus, respiratory syncytial virus, influenzavirus A and B, parainfluenzavirus, adenovirus, and *Mycoplasma pneumoniae*.[17-19]

3.2.6 Statistical analyses

Assuming an infection rate of 0.9 episodes per person per year and a 25% reduction in incidence, sample size calculations showed that with a power of 80% and alpha of 0.05 (one-sided), 220 participants in the vitamin E and 220 individuals in the multivitamin and mineral group should be included. Power was regarded sufficient by including more than 300 participants in both groups, and infection rate was 1.59 per person per year. Although the initial sample size was based on an one-sided test on the assumption that effects would only be seen in one direction, after the study was completed the need for two-sided tests became evident. P values are therefore based on two-sided tests.

An asymptomatic period of at least seven days was required before a subsequent episode was recognized as a new infection. Participants were considered at risk of a new infection during the entire follow-up minus the duration of each illness episode, and minus seven days following each episode. Data analysis was performed according to intention to treat (i.e. based on all participants as randomized). A per protocol analysis was also performed but did not substantively change the study results.

First, data were analyzed by four treatment groups separately. Second, after evaluating possible interactions, data were analyzed according to the 2*2 factorial design.

Continuous data are expressed as mean (SD) and compared using analysis of variance (ANOVA). Total carotenoid concentrations, total illness-duration, and the number of symptoms were log-transformed to account for nonnormality before ANOVA was performed and p values were obtained from ANOVA with log-transformed values. Frequencies, including percentages, were calculated for categorical data and these variables were compared by χ^2 tests. We used a Poisson regression model with number of episodes as the dependent variable, treatment group as the independent variable, and log-person time as the offset included in the model. P values less than 0.05 were regarded as statistically significant. Analyses were performed using SAS statistical software version 6.12 (SAS Institute Inc, Cary, NC).

3.3 RESULTS

Baseline characteristics and plasma antioxidant-vitamin concentrations of the 652 participants were similar across groups (Table 3.1). Only two percent of the participants lived in homes for the elderly. We therefore consider our study population to be noninstitutionalized. In total, 105 (16%) of 652 participants discontinued the intervention (Figure 3.1).

At baseline 40 (6%) and one (0.2%) of the 652 individuals had suboptimal ascorbic acid and alpha-tocopherol concentrations, respectively. After intervention, ascorbic acid was suboptimal in four (1.3%) of 300 participants.

Table 3.1 Baseline characteristics and plasma vitamins of elderly participants by treatment*

Characteristics	Multivitamins-minerals (n=163)	Vitamin E (n=164)	Multivitamins-minerals plus vitamin E (n=172)	Placebo (n=135)
Male	88 (54)	81 (49)	81 (47)	75 (49)
Age (years), mean (SD)	73.1 (7.0)	73.1 (6.2)	73.5 (7.3)	73.4 (7.0)
Body-mass index (kg/m^2), mean (SD)	27.5 (3.4)	27.0 (3.6)	27.5 (3.7)	27.7 (3.6)
Chronic obstructive pulmonary disease	15 (9)	19 (12)	17 (10)	15 (10)
Asthma	3 (2)	5 (3)	5 (3)	3 (2)
Cardiovascular disease	17 (10)	25 (15)	25 (15)	26 (17)
Diabetes mellitus	10 (6)	17 (10)	10 (6)	13 (9)
Rheumatism	7 (4)	5 (3)	4 (2)	11 (7)
Allergy†	34 (21)	32 (20)	32 (19)	25 (16)
Current smoker	9 (6)	21 (13)	14 (8)	14 (9)
Former smoker	81 (50)	79 (48)	89 (52)	75 (49)
Influenza vaccination in 1998‡	124 (78)	126 (79)	122 (72)	103 (71)
History of dietary supplements	63 (39)	67 (41)	69 (40)	56 (37)
Plasma vitamins, μmol/L				
Ascorbic acid, mean (SD)	48.3 (21.3)	49.3 (21.2)	49.5 (21.3)	51.2 (22.2)
Retinol, mean (SD)	2.04 (0.47)	1.97 (0.50)	2.05 (0.44)	1.98 (0.46)
Total carotenoids, median (IQR)	0.44 (0.30-0.65)	0.46 (0.30-0.69)	0.49 (0.33-0.73)	0.44 (0.28-0.70)
α-Tocopherol, mean (SD)	29.4 (7.3)	28.6 (6.2)	29.9 (6.9)	29.1 (6.3)
Cholesterol adjusted α-tocopherol, mean (SD)	4.9 (0.8)	4.9 (1.0)	4.9 (0.8)	4.9 (0.9)
γ-Tocopherol, mean (SD)	2.7 (1.2)	2.6 (1.2)	2.6 (1.2)	2.5 (0.9)

*All data are presented as number (percentage) unless otherwise indicated. SD: Standard deviation; IQR: Interquartile range.

†Allergy against house-dust mite and feces, pollen grains, domestic pets, or molds.

‡Data are missing in four participants in the multivitamin group, five in the vitamin E group, three in the multivitamin-mineral plus vitamin E group, and seven in the placebo group.

After treatment, ascorbic acid, total carotenoids, alpha-tocopherol, and cholesterol-adjusted alpha-tocopherol levels increased significantly in the multivitamin-mineral and multivitamin-mineral plus vitamin E group, while gamma-tocopherol decreased significantly. In the vitamin E group, alpha-tocopherol and cholesterol-adjusted alpha-tocopherol levels increased significantly, while gamma-tocopherol decreased significantly. In the placebo group, none of the measured vitamins changed significantly.

The median follow-up duration was 441 days in each group, representing complete follow-up for 15 months in 84% and including at least three winter months (October-February) for 92% of the participants. Of the 652 participants, 443 (68%) recorded a total of 1024 acute respiratory infection episodes. The study nurse received by telephone 763 (74.4%) of 1024 reports from 381 (86.0%) of 443 participants. Nearly all (99.2%; 757/763) reports were evaluated as acute respiratory infection, the symptoms meeting the criteria and being distinguishable from allergies.

Infection was microbiologically confirmed in 62 (58%) of 107 of the symptomatic periods. In only four (4%) of 91 matched asymptomatic participants was a pathogen identified. The relatively high percentage of microbiological substantiation during the symptomatic periods supports the quality of the self-reported infections.[20;21] A mean of 1.59 episodes was recorded per person per year. The multivitamin-mineral group had 240 episodes with 71% of the participants experiencing at least one episode, the vitamin E group had 280 episodes among 86%, the multivitamin-mineral plus vitamin E group had 274 episodes among 66%, and the placebo group had 230 episodes among 67%.

To assess treatment effect, we first analyzed incidence and severity of the four treatment groups separately (Table 3.2). The only significant difference among the treatment groups was the percentage of participants who experienced restriction of activity, which was significantly lower in the multivitamin-mineral group compared with placebo.

Table 3.2 Effect of four treatments on incidence and severity of acute respiratory infections in elderly participants*

Variable	Multivitamins-minerals (n=163)	Vitamin E (n=164)	Multivitamins-minerals plus vitamin E (n=172)	Placebo (n=153)
Mean incidence of infections per year	1.48	1.73	1.63	1.53
Incidence of infection, rate ratio (95% CI)	0.96 (0.75-1.24)	1.13 (0.88-1.44)	1.06 (0.83-1.35)	1.00
Severity†				
No. with ≥1 infection	116	112	114	102
Total illness duration of sum of respiratory infection, median (IQR), days	14 (6-28)	19 (10-35)	19 (8-38)	14 (6-30)
No. of symptoms, median (IQR)	5 (2-7)	5 (3-8)	6 (4-8)	4 (3-8)
Fever, no. (%)	29 (25.0)	42 (37.5)	41 (36.0)	26 (25.5)
Restriction of activity, no. (%)‡	40 (34.8)§	54 (49.5)	62 (54.9)	48 (48.5)
Episode related medication, no. (%)	35 (30.2)	30 (26.8)	40 (35.1)	34 (33.3)

*CI: Confidence interval; IQR: Interquartile range.
†Analysis assessing severity was performed in participants who experienced at least one respiratory infection.
‡Restriction of activity includes staying in bed, staying at home, not able to do daily activities. Data are missing in one participant in the multivitamin-mineral group, three in the vitamin E group, one in the multivitamin-mineral plus vitamin E group, and three in the placebo group.
§p= 0.04 compared with placebo.

Second, the 2*2 factorial design was used to obtain a more stable estimate of incidence and severity. Neither incidence nor severity was significantly different between the multivitamin-mineral and the no-multivitamin-mineral groups (Table 3.3).

Incidence was not significantly different between the vitamin E and no-vitamin E groups. However, severity tended to be greater in the vitamin E group. Among participants receiving vitamin E and experiencing an infection, total illness-duration and total number of symptoms were significantly higher, and fever and restriction of activity occurred more frequently, than those not receiving vitamin E (Table 3.4). Furthermore, when one-sided tests were used as

originally planned, no p values were significant for any of the tests except for the effect of vitamin E on illness severity. For all microorganisms demonstrated, frequency was not different among the four treatment groups (Table 3.5). After study completion, participants completed a questionnaire asking what they thought the supplemental vitamins contained. Of the 652 participants, 437 (67%) had no idea what the capsule contained, 169 (26%) had the wrong idea, and 46 (7%) were correct.

Table 3.3 Effect of daily multivitamin-mineral supplementation on incidence and severity of acute respiratory infections in elderly participants*

Variable	Multivitamins-minerals (n=335)	No multivitamins-minerals (n=317)	P value
Mean incidence of infections per year	1.55	1.63	
Incidence of infection, rate ratio (95% CI)	0.95 (0.75-1.15)	1.00	0.58
Severity†			
No. with ≥1 infection	230	214	
Total illness duration of sum of respiratory infection, median (IQR), days	16 (7-34)	16 (7-32)	0.67
No. of symptoms, median (IQR)	5 (3-8)	5 (3-8)	0.77
Fever, no. (%)	70 (30.4)	68 (31.8)	0.76
Restriction of activity, no. (%)‡	102 (44.7)	102 (49.0)	0.37
Episode related medication, no. (%)	75 (32.6)	64 (29.9)	0.85

*CI: Confidence interval; IQR: Interquartile range.

†Analysis assessing severity was performed in participants who experienced at least one respiratory infection.

‡Restriction of activity includes staying in bed, staying at home, not able to do daily activities. Data are missing in two participants in the multivitamin-mineral and in six in the no multivitamin-mineral groups.

Table 3.4 Effect of daily vitamin E supplementation on incidence and severity of acute respiratory infections in elderly participants*

Variable	Vitamin E (n=336)	No vitamin E (n=316)	P value
Mean incidence of infections per year	1.68	1.51	
Incidence of infection, rate ratio (95% CI)	1.12 (0.88-1.25)	1.00	0.21
Severity†			
No. with ≥1 infection	226	218	
Total illness duration of sum of respiratory infection, median (IQR), days	19 (9-37)	14 (6-29)	0.02
No. of symptoms, median (IQR)	6 (3-8)	4 (3-8)	0.03
Fever, no. (%)	83 (36.7)	55 (25.2)	0.009
Restriction of activity, no. (%)‡	116 (52.3)	88 (41.1)	0.02
Episode related medication, no. (%)	70 (31.0)	69 (31.7)	0.84

*CI: Confidence interval; IQR: Interquartile range.
†Analysis assessing severity was performed in participants who experienced at least one respiratory infection.
‡Restriction of activity includes staying in bed, staying at home, not able to do daily activities. Data are missing in four participants in both the vitamin E and in the no vitamin E groups.

3.4 DISCUSSION

This randomized placebo-controlled trial demonstrates that long-term daily supplementation with a physiological dose of multivitamins and minerals or with 200mg of vitamin E did not lower incidence and severity of acute respiratory infections in noninstitutionalized elderly persons. However, among persons experiencing an infection, those individuals who received vitamin E instead had longer total illness duration, more symptoms and a higher frequency of fever and restriction of activity.

Table 3.5 Frequency of microorganisms demonstrated in a subsample of 107 elderly participants per treatment group*

Variable	Multivitamins-minerals (n=20)	Vitamin E (n=29)	Multivitamins-minerals plus vitamin E (n=37)	Placebo (n=21)
Not confirmed	7 (35)	16 (55)	11 (30)	11 (52)
Rhinovirus	7 (35)	6 (21)	15 (41)	6 (29)
Coronavirus	6 (30)	4 (14)	4 (11)	4 (19)
Influenza virus A	1 (5)	1 (3)	3 (8)	0
Influenza virus B	0	0	2 (5)	0
Enterovirus	0	1 (3)	1 (3)	0
Parainfluenza virus	0	1 (3)	1 (3)	0
Respiratory syncytial virus	0	0	0	0
Adenovirus	0	0	0	0
Mycoplasma pneumoniae	0	0	1 (3)	0

*No. (%). In one participant form the multivitamin-mineral and one from the multivitamin plus vitamin E group, two pathogens were detected. Percentages do not, therefore, sum to 100%.

In our trial, 94% of the participants met the compliance criteria of 80% capsule intake. Accordingly, the multivitamin-mineral and vitamin E group showed a large increase in plasma vitamin concentrations, whereas this was not the case in the placebo group. Baseline characteristics were well balanced across groups. Assessment of infectious disease was based on self-report, which may have led to misclassification. However, such misclassification would have been nondifferential (i.e. similar for all groups), resulting in possible underestimation of the treatment effect. We tried to assess the outcome as accurately as possible: a prospective diary, telephone calls, home-visits, measuring rectal temperature, plus microbiological and serological testing in a sample. This method of assessing infection has been used before and follows widely accepted criteria.[3;4;7;8] An infection was confirmed in

58% of the symptomatic periods. This percentage is high compared with other studies in which a general practitioner or study nurse evaluated symptoms.[20;21] Therefore, the outcome assessment in our study seems to have been reasonably accurate. Finally, one may argue that an asymptomatic period of seven days is inadequate to discriminate between exacerbation of previous infections and new episodes. Although arbitrarily chosen, this period is considerably longer than asymptomatic periods of previous studies.[22;23]

Past studies of multivitamin and mineral supplementation in noninstitutionalized elderly persons addressed incidence and duration of infectious diseases in general.[3;5] Supplementation trials that specifically focused on incidence of acute respiratory infections have been performed only in institutionalized elderly patients.[8] In the latter intervention study, incidence was not reduced, consistent with our findings. On the contrary, Chandra[3] reported a decreased duration of infectious diseases in noninstitutionalized elderly individuals. However, in that study the proportion of individuals with suboptimal blood vitamin concentrations was much higher. Since Girodon[8] did not observe a lower incidence in institutionalized elderly individuals, it may not be surprising that multivitamin and mineral supplementation in our population did not decrease incidence of acute respiratory infections. The low percentage of participants having suboptimal micronutrient status may reflect a relatively well-nourished population and may explain the lack of a treatment effect. The rationale for the selection of noninstitutionalized elderly persons was based on an improved immune response after multivitamin and mineral supplementation in such populations[3] although this may not necessarily translate to a hard endpoint such as respiratory infections. It is conceivable that particular subgroups of elderly persons who have suboptimal micronutrient concentrations might benefit from additional dietary supplementation.

The only previous studies of vitamin E supplementation in noninstitutionalized elderly persons were intended to measure immune response and assessed infectious diseases only as a

secondary outcome. A lower incidence of all infections was reported in noninstitutionalized elderly individuals,[4] while Harman and White Miller[6] observed no effect on incidence of respiratory infections in institutionalized elderly patients. Although not statistically significant, a higher incidence of upper respiratory tract infection in those supplemented with 50 or 100mg of vitamin E was observed by Pallast et al.[24] We could find no published data on vitamin E supplementation and the severity of respiratory infections.

Many investigators have reported that supplementation with vitamin E improves immune response by enhancing lymphocyte proliferation and interleukin-2 production and by decreasing prostaglandin E2 production by affecting cyclooxygenase activity.[4;9] Others have shown no positive relation between vitamin E and immune indices.[25;26] It should be realized that our observed effect of vitamin E on severity of illness might even reflect a more effective immune response.

Vitamin E may improve immunity by being converted into an alpha-tocopheroxyl radical. This radical can act as pro-oxidant unless it is reduced by ascorbic acid or glutathione.[27;28] The pro-oxidant mechanism of vitamin E has not yet been thoroughly assessed.[28] Possibly, a balance between antioxidants is important in the pro-oxidant role of vitamin E. An imbalance may be more pronounced after long-term supplementation with one nutrient administered in amounts much higher than the RDA level. Azzi and Stocker[27] suggest that the antioxidant effect of vitamin E is not the primary action of this vitamin. Recently, inhibitory effects of vitamin E on protein kinase C and Glutathione S-transferase π have been reported.[27;29]

Most previous studies suggest a beneficial effect of multivitamin, mineral, and vitamin E supplementation on immune response. From a public health point of view, studying incidence of infections, especially the frequent respiratory infections, has much greater relevance. Studies have reported that 50% of elderly people used dietary supplements,[30] with multivitamin, mineral, and vitamin E supplements being the most common.[30;31] It would be worthwhile to study the effect of multivitamins and minerals in elderly people with

suboptimal plasma concentrations of vitamins. If our results are confirmed and vitamin E exacerbates respiratory infections, elderly people, especially those who are already well-nourished should be cautious about taking vitamin E supplements.

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4

Plasma carotenoid status in relation to incidence and severity of acute respiratory infections in elderly people

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ABSTRACT

A high plasma carotenoid status could improve immune response and result in a decreased risk of infectious diseases. Acute respiratory infections are the most frequent of all infectious diseases. Elderly people show an increased risk of respiratory infections and their complications. However, data on the relation of plasma carotenoid status with acute respiratory infections are scarce and not existing for elderly people.

Therefore, we investigated the relation of plasma carotenoid status of six major carotenoids, i.e. beta-carotene, alpha-carotene, beta-cryptoxanthin, lycopene, lutein, and zeaxanthin with the incidence and severity of acute respiratory infections in elderly people. We used the baseline data from an intervention trial. Participants were 652 noninstitutionalized persons (60-95 years) enrolled via two community-based sampling strategies in the Wageningen area of The Netherlands between 1998-1999. Plasma carotenoid status parameters were divided into quartiles, the lowest being the reference. Frequency and severity of episodes during the past year, i.e. presence of fever, staying in bed, medical consultation, and episode-related medication, were self-reported by means of a questionnaire. On average 1.62 episodes per person were recorded. This incidence was in line with prospectively assessed incidences reported in studies in which incidences were effectively validated by nurse or general practitioner assessment, and microbiological analyses. This supports the quality of the self-report. Incidence rate ratio (95% confidence interval) of acute respiratory infections at high beta-carotene status was 0.71 (0.54-0.92). No inverse relation was observed between beta-carotene and illness-severity. Alpha-carotene, beta-cryptoxanthin, lycopene, lutein, and zeaxanthin were not related to incidence or severity of the infections. We conclude that elderly persons with a high plasma beta-carotene have a lower occurrence of acute respiratory infections.

4.1 INTRODUCTION

Elderly people are at high risk of morbidity and mortality from infections, especially the frequently occurring respiratory infections.[1] On average, community-dwelling elderly people suffer from one to two acute respiratory infections per year.[2;3] Observational studies revealed that persons taking diets high in carotenoids seem to have a more optimal immune response. This finding led to the suggestion that the antioxidant properties of carotenoids might help protect human immune cells from oxidative damage.[4-6] However, several human supplementation trials did not corroborate this.[7-9] Beta-carotene, alpha-carotene, beta-cryptoxanthin, zeaxanthin, lycopene, and lutein comprise approximately 90% of the total plasma pool of carotenoids.[10;11] Up to now, beta-carotene has been the most extensively studied carotenoid. Beta-carotene might be beneficial in cellular immunity by reducing oxidative damage and by modulating the production of prostaglandin E2.[4] Information on other main plasma carotenoids in relation to immune response is scarce.[7;12;13] Although hard endpoints such as infectious diseases have much greater public-health relevance than immune function, they have hardly been the subject of study until now. So far, the only study on beta-carotene and common cold incidence in adult male smokers showed no effect, while severity was not measured.[14]

The aim of our retrospective study was to investigate the relation between plasma carotenoid status and acute respiratory infections during the previous year in elderly persons. We investigated incidence and illness-severity in relation to beta-carotene, alpha-carotene, beta-cryptoxanthin, lycopene, lutein, and zeaxanthin.

4.2 METHODS

4.2.1 Subjects

Subjects were approached to participate in a 15-months intervention trial investigating the effect of daily vitamin E (200mg) or multivitamin-mineral (physiological doses) supplementation on acute respiratory infections. The study design has been described in detail previously.[3] We used the baseline information of our study population for this retrospective study concerning carotenoid status and respiratory infections in the previous year. The study population included 652 participants: 325 men and 327 women. All persons were 60 years or older, with an average age of 73 years. Only two percent of the subjects lived in homes for the elderly. We therefore consider our study population to be noninstitutionalized. Participants had no history of cancer, liver disease, or fat malabsorption during five years prior to enrollment. A questionnaire was used to ask for nutritional supplementation during the previous year. Written informed consent was obtained from all participants before participation. The medical ethics committee of the Wageningen University, The Netherlands, approved the research protocol.

4.2.2 Respiratory infections

The main outcomes were incidence and severity of acute respiratory infections during the previous year. At time of blood collection, which was between September 1, 1998 and March 23, 1999, a detailed questionnaire about respiratory infections during the previous year was filled out by all participants with help of a research assistant. Therefore, the recorded infections took place between 1997-1998. Participants were asked about the frequency of common cold, flu, pneumonia, sore throat, and pain in facial sinuses. These different types of infection are simply referred to as ‘acute respiratory infection’. For each past episode of acute

respiratory infection, information indicating illness-severity, i.e. presence of fever, staying in bed, medical consultation, and use of medication, were also recorded in the questionnaire.

4.2.3 Carotenoid analysis

Blood samples were drawn between September 1, 1998 and March 23, 1999 to determine the plasma concentrations of cis-beta-carotene, trans-beta-carotene, alpha-carotene, beta-cryptoxanthin, lycopene, lutein, and zeaxanthin. The beta-carotene concentration was calculated from the sum of cis-beta-carotene and trans-beta-carotene values. If the cis-beta-carotene values were below detection limit (n=269), the trans-beta-carotene value was taken. Concentrations of retinol, alpha-tocopherol, and ascorbic acid were also determined. Samples were collected between 8.30-11.00 AM. A light breakfast, without salads, fruit or fruit juices, was allowed before sampling. None of the subjects had been taking any nutritional supplementation two months prior to blood drawing. Plasma was immediately stored on ice in a closed box, and, within six hours of blood collection, stored at –80°C. The reversed-phase high-pressure liquid chromatography method was used to analyze fat-soluble vitamin concentrations.[15;16] Ascorbic acid concentration was obtained via standard procedures and assessed by fluorimetric assay.[17] Coefficients of variation, showing the reproducibility of results, were 4.3% for beta-carotene, 9.4% for alpha-carotene, 4.2% for beta-cryptoxanthin, and 2.1% for lycopene, whereas coefficients of variation were not available for lutein and zeaxanthin.[15]

4.2.4 Statistical analyses

Participants were divided into quartiles of plasma carotenoid concentrations. The two middle quartiles, i.e. p25-p75, were collapsed and referred to as ‘intermediate carotenoid status’. The low carotenoid class was taken as the reference group. Frequencies including percentages

were calculated for categorical data and were compared by χ^2 test or Fisher's exact test. Continuous variables were compared by analysis of variance for unbalanced data by the SAS procedure GLM (SAS version 8, SAS Institute, Cary, N. Carolina, USA) and were expressed as mean and the corresponding standard deviation (SD).

To calculate incidence rate ratios (IRR) of the infections, a Poisson regression model was used with the number of episodes as the dependent variable and the carotenoid status as the independent variable included in the model. Because some carotenoids possess pro-vitamin A activity, the relation between vitamin A (retinol) and incidence of the infections was assessed. Poisson regression was also used for additional analyses to investigate the relative importance of the carotenoids that were related to the incidence of the infections. Those carotenoids were entered as covariates into one model.

Logistic regression was used to calculate adjusted odds ratios and the corresponding 95 percent confidence intervals (95% CI) for the severity-outcomes. Illness-severity was assessed in participants who experienced at least one acute respiratory infection.

The variables age, body-mass index (BMI), self-rated health (score 1-10, 10 indicating highest self-rated health), retinol status, alpha-tocopherol status, ascorbic acid status, sex, chronic obstructive pulmonary disease (COPD), asthma, influenza vaccination, history of nutritional supplementation, and smoking (current, former, never) were evaluated for confounding and effect modification. If variables were related to the carotenoid status and to the incidence of infection, they were entered into the model. Depending on the carotenoid investigated, sex, age, BMI, self-rated health, and COPD turned out to be confounders. Inclusion of age or sex into the models precluded adjustment for BMI, because of multicollinearity between sex or age and BMI. No effect modifiers turned out to influence the relations. P values less than 0.05 were regarded as statistically significant.

4.3 RESULTS

Population characteristics and plasma (pro-) vitamin concentrations of participants at high, intermediate, and low beta-carotene status are described in Table 4.1. Similar characteristics were observed for the alpha-carotene, beta-cryptoxanthin, lycopene, lutein, and zeaxanthin groups, and are therefore not shown. Correlations between concentrations of the different carotenoids ranged from 0.19 to 0.78. All participants were asked to refrain from nutritional supplementation two months prior to blood drawing. Of the 652 participants, 5.2% used multivitamin-mineral supplementation, 0.2% vitamin A or AD, 3.5% vitamin C, and 1.2% vitamin E during the total previous year of illness-assessment in the questionnaire.

In total 1053 acute respiratory infections were reported by 417 subjects; 235 (36%) of the participants did not suffer from the infections during the previous year. On average, 1.62 episodes were recorded per person per year. A significant inverse relation was observed between beta-carotene status and the incidence rate, but not severity, of the infections (Table 4.2). We observed a tendency for a similar relation between the incidence rate of the infections and alpha-carotene and beta-cryptoxanthin status. Incidence rate ratio (95% CI) at high and intermediate status was 0.79 (0.60-1.02) and 0.82 (0.66-1.01) for alpha-carotene, and 0.83 (0.64-1.07) and 0.78 (0.63-0.97) for beta-cryptoxanthin, respectively. When combinations of beta-carotene, alpha-carotene, and beta-cryptoxanthin were entered into one model, beta-carotene turned out to have the greatest relative importance (data not shown). No significant relation was observed between the parameters indicating illness-severity and alpha-carotene, and beta-cryptoxanthin status. P’s of tests for trend for the severity parameters ranged from 0.14 to 1.00 for alpha-carotene, and from 0.11 to 0.85 for beta-cryptoxanthin.

Table 4.1 Population characteristics and plasma (pro-) vitamin concentrations of 652 Dutch elderly persons by beta-carotene status

Beta-carotene	High (n=162)	Intermediate (n=325)	Low (n=165)
Population characteristics, %			
Men	37.0	47.1	67.9
Age (years), mean (SD)	74.0 (7.1)	73.6 (6.9)	72.2 (6.7)
Body-mass index (kg/m^2), mean (SD)	25.9 (3.3)	27.5 (3.4)	28.4 (3.7)
Self-rated health (score 1 (lowest) - 10), mean (SD)	7.6 (0.9)	7.5 (1.1)	7.2 (1.2)
Chronic obstructive pulmonary disease	4.3	9.5	17.0
Asthma	1.9	2.8	1.8
Allergy*	17.3	20.0	18.2
Current smoker	8.0	9.9	14.6
Influenza vaccination	75.2	74.9	75.2
Plasma vitamins, μmol/L, mean (SD)			
Beta-carotene	0.83 (0.33)	0.40 (0.09)	0.18 (0.05)
Alpha-carotene	0.15 (0.12)	0.07 (0.04)	0.04 (0.02)
Beta-cryptoxanthin	0.43 (0.33)	0.30 (0.21)	0.18 (0.13)
Lycopene	0.43 (0.27)	0.28 (0.19)	0.19 (0.14)
Lutein	0.32 (0.16)	0.25 (0.12)	0.21 (0.11)
Zeaxanthin	0.07 (0.03)	0.06 (0.03)	0.05 (0.02)
Retinol	1.98 (0.45)	2.03 (0.46)	2.01 (0.50)
Alpha-tocopherol	31.4 (7.1)	29.4 (6.1)	26.9 (6.6)
Ascorbic acid	55.0 (20.4)	49.2 (20.8)	44.8 (22.8)

SD: Standard deviation.

*Allergy against pollen grains, domestic pets, or house-dust.

The incidence rate ratios (95% CI) at high and intermediate concentrations were 1.24 (0.96-1.61) and 1.11 (0.89-1.40) for lycopene status; 1.13 (0.86-1.48) and 1.10 (0.88-1.40) for lutein status; and 1.06 (0.83-1.35) and 0.92 (0.74-1.15) for zeaxanthin status. Correspondingly, no significant relation was observed between the parameters indicating infection-severity and

lycopene, lutein, and zeaxanthin status. P's of tests for trend for the severity parameters ranged from 0.36-0.94 for lycopene; from 0.66-0.74 for lutein; and from 0.70-0.97 for zeaxanthin. Additional analysis to investigate the relation between retinol (vitamin A) and the incidence of acute respiratory infections, showed that the incidence rate ratio (95% CI) was 1.00 (0.77-1.30) for high versus low retinol status and 0.98 (0.79-1.24) for intermediate versus low retinol status.

Table 4.2 Incidence and severity of acute respiratory infections according to plasma beta-carotene status in 652 Dutch elderly persons from 1997-1998

Beta-carotene Outcomes*	High (n=162)	Intermediate (n=325)	Low (n=165)	P for trend
Incidence rate (per person per year)	1.66	1.73	2.34	
Incidence rate ratio (95% CI)	0.71 (0.54-0.92)	0.74 (0.60-0.91)	1.00	0.01
Fever†	0.83 (0.44-1.55)	1.07 (0.64-1.79)	1.00	0.56
Staying in bed†	0.74 (0.38-1.44)	1.20 (0.71-2.03)	1.00	0.41
Medical consultation†	1.27 (0.62-2.61)	1.44 (0.79-2.64)	1.00	0.53
Episode-related medication†	1.33 (0.73-2.43)	1.60 (0.96-2.68)	1.00	0.38

CI: Confidence interval.

*All outcomes were corrected for sex, age, self-rated health, and chronic obstructive pulmonary disease.

†Adjusted odds ratios (95% CI) of parameters indicating infection-severity, i.e. fever, staying in bed, medical consultation, and use of medication, were assessed in participants who experienced at least one respiratory infection. These were 103 subjects in the high, 206 in the intermediate, and 108 in the low beta-carotene group.

4.4 DISCUSSION

This observational study demonstrates that elderly persons with a high plasma beta-carotene status have a lower occurrence of acute respiratory infections, whereas not a lower illness-severity. No relation was observed between plasma concentrations of alpha-carotene, beta-cryptoxanthin, lycopene, lutein, and zeaxanthin and the incidence plus severity of acute respiratory infections.

In the following, we will address possible threats to the internal validity in this study. Error in the assessment of both exposure and outcome may have led to information bias. Participants and investigators were unaware of the carotenoid status at the time of outcome assessment, which makes differential error in the assessment of respiratory infections unlikely. Any error in the assessment of carotenoid status and infectious diseases is expected to have been nondifferential and could therefore only have led to underestimation of the association.

One may question whether the plasma carotenoid status of elderly people is stable over an one-year period. Others showed that serum carotenoids were highly reproducible between one- and two year repeated measurements.[18] We therefore have no reason to question the representativeness of the observed carotenoid status for the previous year. Incidence in this retrospective study was in line with incidences reported in prospective studies in which incidences were effectively validated by nurse or general practitioner assessment, or by microbiological analyses.[2;3;19] Although we cannot rule out non-differential error of the self-recorded infections, e.g. exacerbations of COPD could have been indistinguishable from the symptoms during a respiratory infection, this supports the accuracy of self-report of infections that occurred during the previous year.

Bias by confounding was appropriately addressed during the data analysis phase. Multivariate analyses were adjusted for several confounders, such as age, sex, COPD, and self-rated health. We cannot rule out that some confounding by variables we did not measure, e.g. intake of fruit and vegetables, and cognitive function, occurred.

Self-selection of elderly participants can have resulted in the enrollment of relatively healthy, well-nourished elderly people. This may have influenced the generalizability of our results, influencing external validity. Besides, if our population reflects a homogeneous population, the range of carotenoid concentrations in our study may have been too small to have an appreciable effect. However, average plasma carotenoid concentrations and corresponding standard deviations as measured in our population were similar to levels in elderly Europeans, except for the average lycopene concentration which was higher in our population.[20]

Human studies on carotenoids and immune response so far focused on beta-carotene. Some studies have shown an enhanced immune response at high plasma beta-carotene status or after beta-carotene supplementation,[4;21-25] whereas others have not.[7-9;22;26] So far, one study investigated acute respiratory infections, i.e. common cold incidence, in relation to both dietary beta-carotene intake, and beta-carotene supplementation in male smokers. High dietary intake was associated with a slightly higher incidence of colds, whereas beta-carotene supplementation had no effect on common cold incidence.[14] Smokers may have a lower plasma beta-carotene status due to cigarette smoke containing many oxidants and free radicals and due to a different lifestyle.[27-29] It is suggested that beneficial effects of carotenoids on immune response could be due to their antioxidant capacity.[4;30] This might especially be important in subgroups, such as smokers and elderly persons because of increased oxidative stress at old age.[31]

We observed a beneficial association only for beta-carotene, whereas not for the other carotenoids investigated. A high plasma beta-carotene status has been associated with

increased lung function.[32] Lung tissue could be the relevant target tissue for respiratory infections and plasma carotenoid concentrations may serve as a more accessible biomarker of carotenoid status of human lung tissue and bronchoalveolar lavage cells.[33;34] Previous research demonstrated quantifiable levels of several carotenoids in human lung tissue, i.e. lutein, cryptoxanthin, alpha-carotene, beta-carotene, and lycopene. The concentration of beta-carotene was shown to be higher compared to the other carotenoids, but it was not reported whether this was significant or not.[35] One might speculate whether our observed favorable association of beta-carotene is due to its higher concentration in human lung tissue.

Beta-carotene is a precursor of retinol. To explore the possibility that retinol is involved in the observed favorable effect, we performed an additional analysis. Plasma retinol status turned out not to be related to infectious disease incidence, which is in agreement with previously performed studies.[25;36] Although we cannot exclude the possibility that beta-carotene is inversely related to the incidence of respiratory illness by influencing retinol status, we suppose that this mechanism is less plausible.

We did not observe a relation between alpha-carotene, beta-cryptoxanthin, lycopene, lutein, zeaxanthin and acute respiratory infections. Alpha-carotene and beta-cryptoxanthin have never been reported in relation to incidence or severity of acute respiratory infections, nor in relation to human immune response. Literature concerning lycopene, lutein, zeaxanthin and immune function is scarce. No clear effects of lycopene, lutein, and zeaxanthin were observed on blood monocytes and T-helper cell activity.[7;12;13] Correspondingly, no effect of tomato juice, with lycopene being the predominant carotenoid, on cell-mediated immunity was shown.[37] Those findings support our null-findings on respiratory infections. In contrast, Watzl et al.[38] showed improved T-lymphocyte function by tomato juice consumption in persons consuming a diet low in carotenoids.

One may question whether our results concerning plasma carotenoids are in fact due to the intake of fruit and vegetables. A change in the fruit and vegetables intake, the main sources of carotenoids, was shown to result in corresponding changes in plasma carotenoids concentrations.[39;40] Correspondingly, dietary intake of antioxidant (pro-) vitamins and blood status have been shown to be closely related,[41;42] whereas others showed poor relations between dietary intake of fruit and vegetables and plasma carotenoid status.[18;43;44] We cannot exclude the possibility that our observed beneficial association has to be ascribed in part or completely to other biologically active compounds found in fruit and vegetables.

Respiratory infections occur most frequent of all types of infections. Our findings emphasize that elderly persons with a high plasma beta-carotene status have a lower occurrence of acute respiratory infections. Future research could address whether supplementation with beta-carotene or with fruit and vegetables will also result in beneficial effects with respect to the infections.

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5

Alcohol, smoking, and physical activity in relation to respiratory infections in elderly people

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ABSTRACT

Elderly people show an increased risk of acute respiratory infections and their complications. This increased susceptibility may be the result of immunosenescence. If lifestyle factors could influence the risk of the infections, this could result in great public health relevance. We investigated the relation between alcohol consumption, smoking, and physical activity with acute respiratory infections. The lifestyle factors were assessed at baseline by means of standardized and validated questionnaires in 652 Dutch elderly persons. Self-reported respiratory infections were assessed from 1998-2000 by nurse telephone contact, and home visits, and were evaluated by microbiological tests in a subset. We detected 1024 acute respiratory infections among 443 participants, the overall incidence rate (IR) was 1.6 infections per person per year. IR ratio (95% confidence interval) of the infections was 1.31 (1.01, 1.70) for occasional (0<glasses/day≤1), 1.22 (0.92, 1.64) for light (1<glasses/day≤3 (men) and 1<glasses/day≤2 (women)), and 1.33 (1.04, 1.83) for moderate/heavy (>3 (men) or >2 (women) glasses/day) alcohol consumption after adjustment for age and sex. Alcohol intake was not related to illness-severity. Smoking and physical activity were not related to the incidence and severity of the infections. All results remained unaltered after adjustment for lifestyle factors and for other potential risk factors for respiratory infections. We conclude that only alcohol intake may be unfavorably associated with the frequency of acute respiratory infections in apparently healthy elderly people.

5.1 INTRODUCTION

Respiratory infections are the most frequent among all types of infections and are a source of substantial morbidity in elderly people.[1] Increased susceptibility to respiratory infections in elderly people may be the result of immunosenescence.[2;3] Lifestyle factors such as alcohol consumption, smoking, and physical activity may influence the risk of the infections.

The effect of chronic and acute alcohol consumption on the immune system has predominantly been investigated in animal and *in vitro* studies. In general, chronic heavy or excessive alcohol use resulted in impaired innate, humoral, and cellular immunity.[4] Correspondingly, an increased risk of respiratory infections is observed at increasing alcohol intake.[5;6] For light to moderate consumption, results have been contradictory in the few epidemiological studies performed so far,[5;7;8] whereas an inverse association between wine consumption and common cold incidence was observed.[8] In general, smokers are at increased risk of acute and chronic respiratory infections, such as pneumonia, chronic obstructive pulmonary disease, and influenzavirus infections, although some studies do not support this hypothesis.[7;9-12] Loss of cilia of the upper respiratory tract and decreased immune function caused by smoking are some possible explanations for the increased risk.[13;14] However, epidemiological research on the relation between smoking and acute respiratory infections in elderly people is scarce. The effect of sports activity on respiratory infections has predominantly been studied in athletes with exhaustive exercise, in general showing an increased risk of infections.[15] Moderate physical activity in general shows a decreased risk of the infections,[15-18] but few studies were conducted in elderly people.[19;20]

A prospective analysis was conducted to investigate how basic habits of life including alcohol consumption, smoking, and physical activity are related to the incidence and severity of acute respiratory infections in apparently healthy elderly people.

5.2 METHODS

5.2.1 Subjects

All participants were approached to participate in an intervention trial investigating the effect of daily vitamin E or multivitamin-mineral supplementation on acute respiratory infections, which has been described in detail elsewhere.[21] Data from that trial were used for this prospective observational analysis. The study population was enrolled between September 1, 1998 and March 23, 1999 and included 325 men and 327 women of 60 years and older living in the Wageningen area of The Netherlands. All individuals were followed for at maximum 15 months. Written informed consent was obtained prior to study participation from all participants. The medical ethics committee of Wageningen University, The Netherlands, approved the research protocol.

5.2.2 Exposure assessment

A questionnaire concerning population characteristics at baseline, including the lifestyle factors alcohol consumption, smoking, and physical activity was filled out by each participant with help of a research assistant.

Habitual alcohol consumption was measured by identifying the kind of alcohol (beer, wine, spirit, sherry/port, and liquor) by a validated questionnaire. To take into account differences in drinking for weekdays, weekends, and social events, the amounts per day, week, month, or year were recorded. Spearman's correlation coefficients of this questionnaire ranged from 0.74-0.87 for validity and 0.83-0.92 for reproducibility.[22] Since a glass of beer, wine, spirit, sherry, port, or liquor contains approximately similar amounts of ethanol, each was treated as a single drink. Because of a larger body size of men, sex was taken into account when defining alcohol categories. The alcohol exposure categories were divided into four groups: 0

(abstainers); 0<glasses/day≤1 (occasional drinking); 1<glasses/day≤3 for men and 1<glasses/day≤2 for women (light drinking); and >3 glasses/day for men and >2 glasses/day for women (moderate/heavy drinking).

All participants were asked about lifetime smoking habits, number of cigarettes, cigars, and/or pipes smoked daily, at which age they started and stopped smoking, as well as information on quitting smoking for a while, but continuing afterwards. Participants were classified as never, former (all had smoked for at least one year), or current smokers.

A standardized questionnaire, shown to be reliable and valid for classifying healthy elderly people into habitual physical activity categories, was used to calculate an activity score.[23] The score was based on both intensity and duration of all daily physical activities, i.e. household, sports and leisure time activities. Participants were divided into quartiles concerning the score, and categorized into three groups: low, moderate, and high, the second and third quartiles being the moderate group.

5.2.3 Disease assessment

Main outcomes were the incidence and severity of acute respiratory infections. A diary was used in which all symptoms were self-recorded by the participants on a daily basis. Each participant received thorough personal instruction from the investigator on using the diary. Respiratory symptoms were regarded as acute respiratory infection if the symptoms had: (1) a sudden onset; (2) a pattern that differed from any usual symptoms; and (3) if one or more of the upper respiratory tract symptoms, i.e. rhinitis, sore throat or cough were present for at least two days.[24] Illness-duration, being an indicator of illness-severity, was calculated from this diary. Accompanying signs indicating illness-severity were also recorded in the diary: (1) fever; (2) symptoms of a lower respiratory tract infection (sputum production, wheezing, pain on respiration); (3) systemic symptoms (malaise, headache, rigors, muscular pain,

perspiration); (4) activity restriction (staying in bed, not being able to do daily activities, staying at home); and (5) other symptoms (tearful eyes, pain in facial sinuses or ear).[1] Participants were requested to report all symptoms of a possible acute respiratory infection by telephone to the study nurse, who checked whether the symptoms met the definition. Rectal temperature was self-assessed with a study-supplied thermometer on all symptomatic days. As a more specific diagnostic test, microbiology by polymerase chain reaction and serology testing was performed in a random subsample of 107 symptomatic case-episodes and 91 asymptomatic control subjects.[25] A nose-throat swab and acute-phase blood sample was taken within three days and a convalescent blood sample after two to four weeks after onset of symptoms. The nose-throat swab and paired blood samples were tested for the nine most common respiratory viruses (including *Mycoplasma pneumonia*): rhinovirus, enterovirus, coronavirus, respiratory syncytial virus, influenzavirus A and B, parainfluenzavirus 1, 2, and 3, and adenovirus.[26-28]

5.2.4 Statistical analysis

An asymptomatic period of at least seven days was required before a subsequent episode was recognized as a new infection. The person time of each participant was calculated by the entire follow-up period minus the total illness-duration, and minus seven days following each episode.

Data were analyzed with SAS statistical software version 8 (SAS Institute Inc, Cary, NC). Continuous baseline data were expressed as mean with the corresponding standard deviation (SD), and were tested with analysis of variance (ANOVA). Frequencies and percentages for categorical baseline data were calculated and tested with χ^2 test or Fisher's exact test.

A Poisson regression model with the lifestyle factor as the independent variable, number of respiratory infections as the dependent variable, and log person time as offset for the model,

was used to calculate the incidence rate ratio (IRR) of respiratory infections. Infection-severity outcomes, i.e. illness-duration, incidence of infections with fever, symptoms of lower respiratory tract infection, systemic symptoms, activity restriction, and other symptoms were assessed in participants that experienced at least one infection. Average total illness-duration per year was expressed as median (25-75 percent ranges), and compared using proc GLM. After checking for potential confounders and effect modifiers, no effect modifiers turned out to modify the relation. Age, sex, and body-mass index (BMI) turned out to be the only confounders in all models. Because BMI showed multicollinearity with age, this variable was not taken as a confounder in the models. Alcohol consumption, smoking, and physical activity were strongly related to each other. Additional adjustment for these lifestyle variables, or for other potential confounders such as chronic obstructive pulmonary disease (COPD), did not change the results. Participants were originally recruited to participate in an intervention trial concerning multivitamin-mineral and vitamin E supplementation. The distribution of the type of supplementation in each alcohol, smoking, and physical activity group was similar. Results did not change after adjusting for the type of supplementation, which was therefore not included in the models. When excluding those persons who were followed for less than one year, results hardly changed. All 652 participants as enrolled at baseline are therefore included in the analyses. P values less than 0.05 were regarded as statistically significant. All reported p values are two-sided.

5.3 RESULTS

Population characteristics for the alcohol consumption, smoking, and physical activity categories are described in Table 5.1. Participants were aged 60-95 years, the average age

being 73.3 years at baseline. Fifty percent was male. Only two percent of the participants lived in homes for the elderly, and we therefore consider our study population to be noninstitutionalized.

The median follow-up period of the total population was 441 days, the minimum and maximum period being one and 458 days, respectively. In total 443 (68%) of 652 participants recorded 1024 acute respiratory infections. The overall incidence of acute respiratory infections was 1.6 per person per year. Eighty-six percent of the participants reported 75% of the infections by telephone to the study nurse. Of these reports, 99.2% was evaluated as acute respiratory infection, the symptoms meeting the criteria and being distinguishable from allergies. Accuracy of self-diagnosis of the infections was demonstrated by a laboratory confirmed infection in 62 out of 107 symptomatic cases (58%), whereas in only four percent (4/91) of the matched asymptomatic subjects a pathogen was identified.[25]

Moderate/heavy alcohol consumers consumed a median of 3.4 drinks with a maximum of 11 drinks per day. Light and occasional drinkers drank a median of 1.7 and 0.3 drinks per day, respectively. In total 19% of the participants did not consume alcoholic beverages. Abstainers were more often women, were older, and smoked less. Moderate/heavy alcohol consumers had 1.71 acute respiratory infections per person per year, light 1.58, occasional 1.68, and abstainers 1.29. Incidence of infection was higher in all alcohol consumers compared to abstainers, although not significantly in the light alcohol consumption category. Illness duration and the other parameters indicating infection-severity were not different between abstainers and other alcohol consumption categories (Table 5.2). Additional adjustment for smoking, physical activity, or other potential confounders did not change the results concerning incidence and severity. For example incidence rates of infection were 1.29, 1.68, 1.57, and 1.72 for abstainers, occasional, light, and moderate/heavy alcohol consumers after adjustment for age, sex, smoking, and physical activity, respectively.

Current smokers have been smoking for an average (SD) of 48.6 (11.0) years. Former smokers had smoked for at least one year and smoked on average (SD) 30.1 (13.5) years. Never smokers were more often women, were older, consumed less alcohol, and were less physically active. Current smokers had 1.61 acute respiratory infections per person per year, former smokers 1.62, and never smokers 1.54. The frequency and severity of the infections were not different between the current, former, and never smokers (Table 5.2). Additional adjustment for for example COPD, or alcohol consumption and physical activity did not change the results.

The range of the physical activity score in the total population was 0.1-36. The less active people performed vigorous activities less than 15 minutes a day, walked less stairs, and generally did not do heavy housework. The more active people of our research population spent several hours per week in vigorous activities such as swimming, cycling, gardening, and gymnastics. People with low physical activity were older, had a lower BMI, smoked less, and were more often vaccinated against influenza virus. Incidence rate of infection was 1.72 in the high, 1.55 in the moderate, and 1.53 in the low activity categories per person per year, the incidence rate ratios not being significantly different. People with moderate physical activity had significantly less infections with fever and activity restriction compared to people with low activity, whereas the other severity-parameters were not different between these two categories. People with high and low physical activity also did not differ in illness-severity (Table 5.2). Additional adjustment for potential confounders did not change the results concerning incidence and severity of the infections. For example, incidence rate ratios were 1.12 at high, and 1.01 at moderate physical activity after adjustment for age, sex, smoking, and alcohol consumption.

5.4 DISCUSSION

In this prospective analysis alcohol consumption was related to an increased incidence of acute respiratory infections. Smoking and physical activity were not related to the incidence and severity of the infections.

The prospective study design ensured that lifestyle factors were assessed before diagnosis of acute respiratory infections was made. This will have prevented differential error in the assessment of lifestyle factors. Our questionnaires for alcohol consumption and physical activity are shown to be valid and reliable for assessing alcohol consumption and physical activity in elderly people.[22;23] However, error in recall and socially desirable answers may probably have resulted in non-differential error, diluting the association.[23] Concerning smoking, a 96.4% agreement was observed between the self-reported smoking status and the status as determined by measuring cotinine levels in serum which is a metabolite of nicotine.[7] We therefore conclude that differential error is not likely, whereas we are not certain that non-differential error in the assessment of lifestyle factors did not occur. This may (partly) explain our null-findings.

A crucial element in this study is the self-report of respiratory infections. Differential misclassification may have occurred if, for example, heavy alcohol consumers over- or underreported their infections, whereas abstainers did not. Because both over- and underreporting of the infections is possible, and such error is difficult to quantify, the net effect of a possible differential misclassification in the assessment of outcome is unknown. Non-differential misclassification may have occurred if, for example, normal ‘daily’ respiratory symptoms were reported as acute respiratory infection. However, we have tried to assess the outcome as accurately as possible: a prospective diary, telephone calls, home-visits,

rectal temperature, and microbiological and serological testing in a subset. Our way of assessing infection has been used before and contains widely accepted criteria.[29-32] Furthermore, infection was laboratory-confirmed in 58% of the symptomatic cases.[25] This percentage is high as compared to other studies in which a general practitioner or nurse performed evaluation of symptoms.[1;33]

Participants were approached to participate in an intervention trial concerning micronutrient supplementation. We cannot exclude that self-selection has resulted in enrollment of relatively health-conscious and healthy people. This may have influenced the generalizability of the results. Studying an elderly population has a number of implications. The older participants may on one hand represent healthy survivors, but on the other hand may be of ill health and for example less able to recall lifetime smoking habits. Although age was taken as a confounder in the models, one may question whether it was taken into account sufficiently for our conclusions. Apart from age, comorbidity, diet, and type and number of social contacts have also been associated with respiratory infections.[29-32;34;35] Comorbidity may especially be crucial in the study of elderly persons. However, the frequency of common diseases or indicators of comorbidity as self-reported in a questionnaire, e.g. diabetes mellitus, high cholesterol level, and high blood pressure, was not different between our lifestyle categories (data not shown). Furthermore, people with cancer and liver, intestinal, and kidney diseases were excluded from participation. Therefore, we suppose that confounding by comorbidity is of minor importance in our analysis. Malnutrition is reported to decrease immune response and malnourished people could therefore be more susceptible to infections.[30;31] However, our study population had an average (SD) body-mass index of 27.4 (3.6) kg/m^2 at baseline and participants gained on average (SD) 1.2 (2.5) kilograms during participation. Furthermore, at baseline only six percent of participants had suboptimal vitamin C and 1.3% had suboptimal vitamin E plasma concentrations.[21] We consider our population as an apparently healthy

elderly population with an adequate nutritional status. A more diverse social network was shown to be associated with greater resistance to upper respiratory illness,[35] a factor we did not take into account. Apart from network diversity, also the number of social contacts may be crucial in the study of a contagious disease. The general idea is that a high number of contacts increases the risk of getting infected. Because the number of contacts is not only related to infection risk but also to lifestyle, confounding by social contacts may occur in observational research such as ours. However, the only study carried out so far on this showed no association between the total number of network members and common cold incidence in adults.[35]

It is suggested that a 'J' shaped relationship reflects the association of immune function with alcohol consumption.[36] If an improved immune function would translate into decreased illness, we would expect to observe a lower incidence of acute respiratory infections in light alcohol consumers. We observed however a linear relationship, which is in line with a study in students,[5] and in male smokers,[37] whereas not with a study in adults showing no relation between alcohol consumption and incidence of common colds.[8] Another study in adults even showed a decreased risk of common cold, but in that study volunteers were artificially challenged with rhinovirus.[7] It is remarkable that none of these studies on alcohol consumption and acute respiratory infections observed a 'J' shaped relationship. The linear relation as observed in our analysis and by some others, might be explained by the fact that alcohol stimulates virus replication, as observed at hepatitis virus and human immunodeficiency virus infections.[38-41]

In our analysis, abstainers were taken as reference. Lifestyle of abstainers could be different from persons that consume alcohol. One may therefore question whether abstainers reflect an adequate reference category. Because we wanted to compare our results to others, we used abstainers as a reference.

We observed no relation between smoking and acute respiratory infections. This is in line with some previous research on influenza and common cold incidence.[10;11] However, the majority observed that smokers had a higher risk of developing acute respiratory infections and complications during such infections.[7;9;11;12;42] The number of current smokers in our study may have been too small to show an effect. Because our study on lifestyle was a secondary analysis, the number of recruited participants was based on sample size calculations for a randomized controlled trial on micronutrient supplementation.[21] If we would have included more individuals, small numbers in the current smoking group could have been overcome.

People with moderate physical activity had less infections with fever and activity restriction, whereas no beneficial associations were observed with the other severity-parameters or with the incidence of acute respiratory infections. We could therefore not conclude that in healthy elderly persons the incidence and illness-duration of acute respiratory infections is negatively associated with moderate physical activity, as was observed in the few studies on exercise and respiratory infections in elderly people.[19;20] However, in those studies, sports activity was taken as the exposure. We used the sum of sports, household, and leisure time activity as exposure, because sports activity was shown to contribute little to physical activity in the aged.[43] This might explain the discrepancy in results.

In conclusion, only alcohol consumption may be unfavorably associated with incidence of acute respiratory infections in apparently healthy elderly people, whereas smoking and physical activity may not. Further research may help to clarify whether the association between alcohol consumption and acute respiratory infections can be partially explained by unmeasured variables, such as the type of alcohol consumption, and social network.

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6

General discussion

Many of the methodological strengths and limitations of the research conducted for this thesis have been considered in the previous chapters. In this chapter, we will continue our discussion with those design issues which are especially critical for the validity of our studies, i.e. study population, treatment, and assessment of exposure and outcome. Furthermore, several types of bias, i.e. selection and information bias, and confounding will be addressed. First, the major findings of the studies described in this thesis will be reviewed. After discussion of the critical design issues and biases, we will present public health recommendations, and suggestions for future research.

6.1 MAIN FINDINGS

The main findings of the studies described in this thesis are summarized in Table 6.1. Our virological characterization showed the importance of rhinovirus infections in elderly people. Also, a few asymptomatic elderly persons were shown to harbour respiratory pathogens and these might therefore serve as a source of infection (Chapter 2). Our randomized controlled trial showed that neither daily supplementation with multivitamins-minerals at doses near the recommended dietary allowance nor with 200mg of vitamin E had a beneficial effect on the incidence and severity of acute respiratory infections in well-nourished elderly people. Instead, we observed unfavorable effects of vitamin E on illness-severity (Chapter 3). In our analysis on carotenoids, a higher plasma beta-carotene status was associated with a lower incidence of acute respiratory infections, whereas such positive associations were not observed for other major plasma carotenoids (Chapter 4). Our analysis on lifestyle factors showed that a higher alcohol consumption may be associated with a higher incidence of the infections, whereas this was not the case for smoking and physical activity (Chapter 5).

Table 6.1 Main findings on incidence and severity of acute respiratory infections investigated in 652 relatively healthy well-nourished Dutch elderly persons

Randomized controlled trial			
Ch. 3:	Multivitamins-minerals	IRR (95% CI) 0.95 (0.75-1.15).	
	Vitamin E	IRR (95% CI) 1.12 (0.88-1.25).	
		Severity :	illness-duration 19 vs 14 days (p=0.02);
			no. of symptoms 6 vs 4 (p=0.03);
			presence of fever 37% vs 25% (p=0.009);
			presence of activity-restriction 52% vs 41% (p=0.02).
Observational analyses			
Ch. 2:	Virology*	58% Laboratory confirmed.	
		Major viruses:	32% rhinoviruses;
			17% coronaviruses;
			7% influenzaviruses.
		Subclinical infections in 4%.	
Ch. 4	Carotenoids	IRR (95% CI)	0.71 (0.54-0.92) high beta-carotene;
			0.74 (0.60-0.91) intermediate beta-carotene.
Ch. 5	Lifestyle factors	IRR (95% CI)	1.33 (1.04-1.83) moderate/high alcohol consumption;
			1.22 (0.92-1.64) light alcohol consumption;
			1.31 (1.01-1.70) occasional alcohol consumption.

IRR: Incidence rate ratio; CI: Confidence interval

*Virology was performed in 97 symptomatic persons experiencing 107 episodes and in 91 asymptomatic persons.

6.2 CRITICAL DESIGN ISSUES

6.2.1 Study population

Initially, we have tried to recruit frail elderly people. We obtained permission for writing invitation letters to or having meetings with the inhabitants of 21 homes for the elderly and sheltered houses. Of the 1.657 persons that were approached, response rate turned out to be 4.1% of whom only about half (47%) met the inclusion criteria. A major reason for not willing to participate was that these relatively frail elderly people did not want to take additional capsules for research purposes on top of their regular medication. The second major reason was that they did not want to be involved in a study with such a long follow-up period. A minor reason was that they thought to be incapable of remembering to fulfill study procedures like filling in a diary. If we would have continued recruiting institutionalized elderly people, enrollment of the required number of participants would have taken many years. To solve the difficulty in recruitment we could have changed our study design, such as (1) supply fortified drinks in stead of capsules; (2) perform weekly telephone calls to check for respiratory illness; or (3) minimize follow-up period. However, weekly telephone calls to all participants were not feasible because of personnel constraints and minimizing follow-up period would have required an even larger number of participants necessary to show an effect. Because other studies showed beneficial effects of micronutrient supplementation on immune function and infectious diseases not only in institutionalized elderly people but also in free-living elderly people, the best solution at that time appeared to be to recruit more healthy noninstitutionalized elderly people. Besides, in 2001 only about five percent of the Dutch elderly population was living in nursing homes and homes for the elderly. Investigating our research question in a noninstitutionalized relatively healthy elderly population could therefore be of even greater public health relevance. This recruitment strategy enabled us to

enroll even more participants in the trial than we needed based on power calculations. Furthermore, comorbidity, mortality, and mental disability are predominantly present in institutionalized elderly people as compared with their peers living noninstitutionalized. Such complicating factors, which are especially important in long-term studies such as ours, were also overcome by choosing a different study population.

The population characteristics and plasma vitamin concentrations at baseline showed that we indeed enrolled a relatively healthy and well-nourished population. This may have reduced the effect of the treatment in our trial, and the generalizability of our results to the elderly population in general. If we would have observed favorable effects, it might have been legitimated to extrapolate the results to the institutionalized frailer elderly population. With our null-findings, such generalization is not possible.

In summary, we were not able to recruit a sufficient number of institutionalized elderly people as initially aimed for. Recruitment of relatively healthy noninstitutionalized elderly people was the best solution at the time of the set-up of the trial. After having interpreted the results, we conclude that this may have diminished the probability of confirming our study hypotheses as described in chapter 1. However, the public health relevance of our findings concerning apparently healthy elderly people may even be greater.

6.2.2 Treatment

Crucial factors concerning our treatment were the dosage of the multivitamin-mineral and vitamin E supplement, and the compliance.

Dosage of multivitamin-mineral supplement

The choice of an appropriate dosage could be based on dose-finding studies with immune response as outcome. We did not conduct such studies to define the most optimal dosage of

the vitamins and minerals. And even though, it would have been questionable if the observed optimal dosage in improving immune function was also the most optimal dosage in reducing the risk of infections. Based on the literature, we chose to supply with a commercially available multivitamin-mineral supplement that included at least those micronutrients that were reported to be (marginally) deficient in (part of) the elderly population, or for which a relation with immune response was reported.[1-5] Our micronutrient dosages were somewhat lower as compared with other trials that showed beneficial effects on immune function or infectious diseases.[6-11] We cannot rule out that the somewhat lower dosages (partly) explain our null-findings. However, the trials that observed beneficial effects on infectious diseases were all performed in people with nutritional deficiencies,[6;7;9-11] and this may be another explanation for the difference in results.

In the course of the follow-up period of a trial, the contents of capsules may change. Storage is therefore also a critical issue, especially in long-term studies such as ours. A pre- and post intervention quality-control showed that the dosages of our treatment were similar after two years of storage. If we would have observed reduced dosages, it would have been unresolved whether this played a role in our null-findings or not.

Dosage of vitamin E supplement

We evaluated the effect of 50 and 100mg of vitamin E on several immune parameters in previous trials. No beneficial effects were observed in apparently healthy elderly people.[12;13] We chose to supply with 200mg of vitamin E, since this dosage was shown to be most optimal in improving immune response in healthy American elderly people.[14] Their beneficial effect on immune response was not confirmed in our trial on infectious diseases. Therefore, the question still remains which dosage of vitamin E would have been most appropriate in reducing the risk of acute respiratory infections. Until now, only one study investigated

infectious diseases as a primary outcome after supplementation with a high dose of vitamin E in humans, showing no effect of 200/400mg in institutionalized patients.[15] Two intervention trials in noninstitutionalized elderly people investigated infectious disease incidence only as a secondary outcome: one showed a non-significant higher incidence of upper respiratory tract infections, whereas the other trial showed a 30% lower incidence.[12;14] The limited information on the mechanism of vitamin E and infectious diseases in humans hampers the definition of the optimal dosage of vitamin E.

As also discussed in chapter 3, we can only speculate about explanations for our unfavorable findings. The beneficial effects of different dosages of vitamin E on immune response were predominantly observed by one research group. The apparent discrepancy of results between their studies and ours might be explained by the fact that alpha-tocopherol concentrations of healthy American elderly people are in general lower compared with their healthy European peers.[14;16] Besides, we cannot rule out that our unfavorable findings on illness-severity might even reflect an increased immune response. Another explanation might be the reduction of plasma gamma-tocopherol by supplementation with alpha-tocopherol, as also observed in our trial. Gamma-tocopherol could exhibit anti-inflammatory activity. A reduction in plasma concentrations of this isomer may have resulted in a reduced immune function and an increased risk of infectious diseases and illness-severity. Although gamma-tocopherol has relatively low antioxidant capacity, and its relevance for immune response or infectious diseases has not been investigated in human trials so far, we cannot exclude that this mechanism has played a role in our findings.[17-19]

Compliance

The returned pill-count indicated a high compliance in our trial. Correspondingly, plasma vitamin concentrations increased significantly in the vitamin E and multivitamin-mineral

groups, whereas not in the placebo group. More important, our increases of vitamin E, C, and beta-carotene were in line with increases observed in several other trials in elderly people after micronutrient supplementation.[10;12-14;20;21] We therefore are confident that compliance was sufficient in our trial. We can only speculate about the factors that contributed to our high compliance. After the trial a questionnaire was filled out by all participants which showed that regular telephone contact, meetings, home-visits, and monthly newsletters meant welcome social activities. Furthermore, for the majority of the subjects participation in scientific research gave them a feeling of being useful for the next generations. Besides, the relatively healthy status of our participants could also have played a role in the high compliance.

Our main conclusion concerning treatment is that the relatively moderate dosages of vitamins and minerals in our multivitamin-mineral treatment may have resulted in null-findings. Concerning vitamin E, limited information on vitamin E and its mechanism with respect to infectious diseases in humans hampers the definition of the optimal dosage of vitamin E.

6.2.3 Assessment of exposure

Plasma carotenoid status

We addressed the etiologic question whether six major plasma carotenoids are related to acute respiratory infections. So far, this was only investigated for beta-carotene in male smokers.[22] One may question whether the plasma carotenoid status adequately reflects the carotenoid status of target tissue, such as lung tissue. Previous studies showed that plasma concentrations of total carotenoids and beta-carotene correlated well with concentrations in bronchoalveolar lavage cells and human lung tissue.[23;24] Plasma carotenoid concentrations may therefore be regarded as suitable biomarkers for carotenoid status of the lung.

It is not clear whether information on plasma carotenoid status can be extrapolated to the intake of certain foods. Some studies showed a significant relation between dietary intake of

fruit and vegetables and plasma carotenoid status, whereas others did not.[25-30] If it would consistently have been shown that this relation is strong, we could have translated our findings into a dietary advise. Because of the inconsistency in the literature, such extrapolation is not possible.

In conclusion, plasma carotenoid concentrations may be regarded as suitable biomarkers for carotenoid status of the lung.

Lifestyle

Self-report in a questionnaire, as used in our analysis on lifestyle, is a cheap and non-invasive method to obtain information and a questionnaire is easily applicable in large epidemiological settings. However, accurate measurement of alcohol consumption, smoking habits, and physical activity is particularly difficult and relying on self-report can result in severe under- or overestimation. This can seriously affect the validity of the results, as will be discussed later in this chapter.

With the difficulties involved in measuring lifestyle factors with a questionnaire, one may question whether the assessment of biomarkers of lifestyle is to be preferred above the assessment of lifestyle by questionnaires. A great advantage of biomarkers is that they are an objective measure not influenced by factors that distort questionnaire information. High-density lipoprotein (HDL)-cholesterol and carbohydrate-deficient transferrin concentrations in blood can, for example, be used as a marker for habitual alcohol consumption. A major disadvantage of HDL-cholesterol is that it is not only influenced by alcohol consumption but also by several other factors, such as fat intake. Furthermore, both markers are not suitable for the assessment of light and occasional alcohol consumption.[31] Valid biomarkers for habitual alcohol consumption were therefore not available.

Expired carbon monoxide and blood cotinine, the major metabolite of nicotine, are markers that reflect the recent smoking status.[32;33] Human toenails grow at a rate of one centimeter every nine to twelve months and human toenail nicotine concentrations reflect therefore a relatively longer cumulative exposure period.[34] We did not know the induction period of smoking, and both recent and lifelong smoking habits could have influenced the risk of acute respiratory infections. A questionnaire assessing both recent and lifelong smoking habits was therefore preferred.

Both acute and chronic habitual sports activity were reported to influence immune response.[35] Our questionnaire on physical activity assessed household, sports, and leisure time activity during the previous year.[36] Recently, a hip-mounted accelerometer was presented as a promising technique to reflect total physical activity. This meter reflects however only recent physical activity. Even more important, accelerometer measurements showed poor correlations with total physical activity and energy expenditure, as assessed by indirect calorimetry, and doubly labeled water method.[37-39] A questionnaire was therefore the best solution to assess habitual physical activity during the past year.

Overall, we conclude that no valid biomarkers to assess alcohol consumption, smoking habits, and physical activity were available to investigate our hypothesis. Therefore, although questionnaires have several limitations, questionnaires were preferred above biomarkers to assess lifestyle factors.

6.2.4 Assessment of outcome

A diary and questionnaire were used to assess the frequency and severity of acute respiratory infections in the prospective (chapter 2, 3, and 5) and retrospective (chapter 4) studies, respectively. In the prospective studies, additional confirmation was obtained by telephone-check and home-visits by the study nurse. Furthermore, objective microbiological and

serological tests were performed in a subset to evaluate the self-report. Such methods of assessing infection have been used before in large epidemiological trials such as ours.[6;14;21;40] We could have chosen to include only laboratory-confirmed infections in our studies, performing microbiological and serological tests in all participants reporting acute respiratory illness. In that case, such analyses should have tested all known respiratory pathogens to reduce the percentage of false negatives. However, this would have involved enormous costs. And even then, the diagnostic deficit, i.e. the percentage of infections without microbiological substantiation, would not have been reduced to zero because of diagnostic failures and unknown pathogens.[41;42] Afterwards, our results showed that symptoms of persons with laboratory confirmed infections did not materially differ from symptoms of persons in whom the infection was not laboratory confirmed. Therefore, if we had chosen to include only laboratory confirmed infections, this would probably have resulted in considerable underestimation of the number of infections. We therefore suppose that, if an appropriate case-definition is used for the self-report, and the self-report is adequately substantiated, self-report is an appropriate method to assess acute respiratory infections in large epidemiological studies such as ours.

Other methods to substantiate the self-report could have been radiological tests of chest and sinuses, assessment of fever and hematological parameters. We chose not to use radiological tests of chest and sinuses to assess the presence of infection because such tests were not feasible in our predominantly noninstitutionalized participants who were living in 3 of the 12 provinces of The Netherlands. In all studies described in this thesis, the presence of fever was self-assessed during the infections with a study supplied thermometer. Because not all acute respiratory infections are accompanied by fever, and fever may have different causes, [43;44] the presence of fever is not an adequate method of assessing acute respiratory infection. Total and differential white blood cell counts, C-reactive protein, and erythrocyte sedimentation rate are

hematological markers that indicate the presence of an infection. Such hematological markers are aspecific, i.e. changes in these markers do not reveal whether they are caused by a respiratory infection.[45-47] But because blood drawing for analysis of hematological parameters can be combined with blood drawing for other purposes, such as serological analysis, hematological markers could be a useful method to obtain additional substantiation on acute respiratory infections.

Overall, we conclude that self-report is an adequate method to assess acute respiratory infections in large epidemiological settings, on the condition that an appropriate case-definition is used, and the self-report is supported by other sources of information.

6.3 BIAS

6.3.1 Selection bias

Selection bias results from procedures used to select subjects, that lead to an effect-estimate among participants of the study population that differs from the estimate that would be obtained from the entire population.[48] In the intervention trial (chapter 3), a random and double-blind treatment allocation was applied to a large number of participants, virtually assuring comparability of the treatment groups. Furthermore, loss-to-follow up was similar between groups. Selection bias will therefore not have threatened internal validity in the intervention trial.

Observational studies (chapter 4 and 5) are sensitive to selection bias. In chapter 4, the low carotenoid categories may comprise people with ill health because of the inverse relation between nutritional status and morbidity.[49] These persons may therefore have been at higher risk of diseases, including infectious diseases. However, persons with low carotenoid

concentrations did not show differences in the prevalence of, for example, diabetes mellitus, rheumatism, high cholesterol level, and high blood pressure. Although selection bias cannot be ruled out, these arguments make such bias less likely.

A different problem may have been reverse causality. This means that the low carotenoid status may not be a cause, but a consequence of greater morbidity in this group. Because exposure and outcome were assessed at the same time, we cannot rule out such type of bias may have occurred in our analysis.

In the observational analysis on lifestyle factors (chapter 5), selection bias is a critical issue. The reference categories, i.e. abstainers, never smokers, and people with low physical activity, may comprise people with a different lifestyle compared to the other categories. On the one hand they may have been of ill health and therefore be at higher risk of acute respiratory infections; on the other hand abstainers, never smokers and people with high physical activity may have been more health conscious and correspondingly be at lower risk. Looking at the prevalence of indicators of morbidity, e.g. high cholesterol level, diabetes mellitus, high blood pressure, these indicators were similar between categories. Thus, although we did not found an indication for selection bias, we cannot ignore the possibility that such bias has occurred.

One might question if we chose the most appropriate reference categories, because of the risk of selection bias. Abstainers were chosen as a reference because we wanted to compare our results to the epidemiological studies that have been performed which all had abstainers as a reference.[9-11;50-52] In the case of smoking, if we would have chosen former smokers as a reference, selection bias could also have occurred, because these persons could have quit smoking because of ill health. Furthermore, chosing smokers as a reference was not ideal, because in our population only a minority of the participants smoked. These arguments make the choice of never smokers as a reference category less problematic. Concerning physical activity, if people with high or intermediate activity would have served as a reference, the risk

of selection bias would not have been reduced. It was therefore a logic decision to have people with low physical activity as a reference.

Corresponding to the analysis on carotenoids, a different problem may have been reverse causality. People could have changed their daily habits of life as a consequence of higher disease. The differences in lifestyle factors as measured in our analysis are in that case not a cause, but a consequence of disease. Such type of bias cannot be ruled out in our analysis on lifestyle.

In conclusion, selection bias was no issue in the trial, whereas we cannot ignore that it may have happened in the observational analyses. Our beneficial finding on beta-carotene, our unfavorable finding on alcohol consumption, and our null-findings on smoking and physical might be explained by selection bias.

6.3.2 Information bias

Information bias occurs when there are errors in the assessment of exposure and outcome. Such errors can be differential, i.e. misclassification of exposure depends on outcome or vice versa, or can be non-differential, i.e. misclassification of exposure does not depend on outcome or vice versa.[48] First, error in the assessment of exposure will be discussed and subsequently error in the outcome assessment.

Exposure assessment error

In the analysis on carotenoids (chapter 4), one may question whether our observed carotenoid status adequately reflected the carotenoid status of the past year, and whether the past year is the relevant induction period of the carotenoids. Serum carotenoids were shown to be highly reproducible between one- and two-year repeat samples.[29] We therefore suppose that our measured carotenoid status adequately reflected the carotenoid status of the past year. The

induction period of at least beta-carotene is probably short, because changes in beta-carotene intake or beta-carotene supplementation were reported to enhance immune response after a relatively short period of time, i.e. several weeks.[53;54] It is therefore plausible that our observation period, i.e. the past year, is the relevant period of our carotenoid status.

As in most studies, some random error in the assessment of plasma carotenoid concentrations is likely and such non-differential misclassification may have resulted in bias to the null.

In the analysis on lifestyle (chapter 5), the prospective study design ensured that lifestyle was assessed before diagnosis of acute respiratory infections. Differential misclassification in the assessment of exposure is therefore not plausible. Non-differential misclassification in the assessment of lifestyle was inevitably present, especially because we were dealing with elderly people. Although others showed that our questionnaires were valid and reproducible,[36;55] some non-differential error in exposure assessment could have resulted in bias to the null.

Overall, differential misclassification in our observational analyses is not likely, although non-differential misclassification could have resulted in bias to the null in both studies.

Outcome assessment error

In all studies as described in this thesis differential and non-differential misclassification in the assessment of outcome is a critical issue. Differential misclassification will not have been an issue in the intervention trial (chapter 3) because personnel was still blinded at the time of outcome assessment. Differential error in the analysis on carotenoids (chapter 4) might have occurred if persons with low carotenoid concentrations, compared to those with intermediate and high concentrations, had a diminished cognitive function resulting in inaccurate remembering of past infections. Although differential misclassification cannot be ruled out, this is not likely because blood drawing and questioning about respiratory infections during

the previous year took place at the same time. In contrast, differential misclassification might have happened in the analysis on lifestyle. Of the lifestyle factors we investigated, bias could especially have influenced the association of smoking with infectious diseases because on the one hand smokers may pay more attention to acute respiratory infections than never smokers may do and could therefore overreport their infections. On the other hand, underreport of the infections may have occurred when smokers evaluated symptoms of acute respiratory infections, such as cough, as their usual cough caused by smoking. Because such error is difficult to quantify, the net effect of this bias is unknown. In conclusion, differential error in outcome assessment is less likely in the micronutrient studies, whereas we cannot ignore that it might have happened in the analysis on lifestyle.

Non-differential misclassification in the self-report of acute respiratory infections can have occurred in all studies as described in this thesis. For example, certain acute allergic reactions, normal daily respiratory symptoms, and exacerbations of COPD may have been reported as acute respiratory infection. Differences in perceiving infections is a potential source of bias in all studies described in this thesis. Especially in our retrospective analysis on carotenoids, impairment of cognitive function may have hampered accurate remembering of acute respiratory infections in the previous year. Non-differential error in outcome assessment can therefore not be ruled out and could have resulted in bias to the null.

6.3.3 Confounding

Several factors are reported to be risk factors for acute respiratory infections, such as nutritional status, smoking, COPD, asthma, and other comorbid conditions. Such factors have to be considered as confounders if they are associated with exposure and if they are not an intermediate step in the causal pathway between exposure and outcome.[48]

In the intervention trial bias by confounding was overcome by a random and double-blind treatment allocation to a large number of participants, resulting in similar characteristics between groups. Although confounding might have occurred by variables we did not measure, e.g. dietary intake, we suppose that such unmeasured variables will also have been similar between groups.

In contrast with the intervention trial, the observational studies are subject to confounding. Bias by confounding was appropriately addressed in the data analysis phase: we adjusted for several confounders in the multivariate analyses, such as age and sex. Potential confounders in the analysis on carotenoids that were not measured were the intake of fruit and vegetables. Such variables may have reduced or increased our observed association. In the analysis on lifestyle, a possible confounder we did not measure was the diversity of the social network, and the number of social contacts. Although the influence of social network on acute respiratory infections is under debate,[56] it could have resulted in some confounding. The individual effect of a lifestyle factor is difficult to measure in observational studies, because they often reflect a behavioral pattern. In our study on lifestyle, adjustment for other lifestyle factors, e.g. adjustment for smoking and physical activity while investigating the association between alcohol consumption and respiratory infections, did not change the results and these variables were therefore not treated as confounders in our analysis.

In conclusion, confounding was no issue in the intervention trial. In the observational analyses on carotenoids and lifestyle, confounding was appropriately addressed during the data analysis phase by adjustment for major confounders in multivariate analyses. However, some residual confounding and confounding by variables we did not measure, could have occurred.

6.4 CONCLUSION

Our major conclusion is that relatively healthy well-nourished elderly people do not benefit from supplementation with vitamin E and multivitamins-minerals in reducing the frequency and severity of acute respiratory infections. Instead, 200mg of vitamin E may have unfavorable effects on illness-severity. We cannot exclude that our unfavorable findings were due to chance. The limited information on the mechanism behind the relation between vitamin E and infectious diseases hampers the identification of the optimal dosage of vitamin E. The relatively low dosages of micronutrients in our multivitamin-mineral supplement might have played a role in our null-findings, although we speculate that the well-nourished status of the population might be of more importance. We doubt whether our conclusions can be extrapolated to elderly people with a suboptimal nutritional status. Although self-report of acute respiratory infections may have led to non-differential misclassification resulting in bias to the null, it is a suitable method if an appropriate case-definition is used, and the self-report is adequately supported by other sources of information.

A high plasma beta-carotene status may reduce the frequency of acute respiratory infections, whereas other major carotenoids may not. We cannot rule out that selection bias (partly) explained our findings.

Increased alcohol consumption may increase the risk of acute respiratory infections, whereas smoking and physical activity may not. However, bias may be responsible for our beneficial findings on alcohol consumption and our null-findings on smoking and physical activity. The influence of lifestyle factors on acute respiratory infections remains therefore subject to debate.

6.5 PUBLIC HEALTH RECOMMENDATIONS

Our randomized controlled trial provides evidence that relatively healthy well-nourished elderly people will neither benefit from supplementation with multivitamins-minerals nor with vitamin E in reducing the risk of acute respiratory infections. We therefore see no reason to recommend relatively healthy well-nourished elderly people to take multivitamins-minerals and vitamin E to reduce the risk of acute respiratory infections.

We cannot be certain that elderly people with suboptimal or even deficient plasma concentrations of several micronutrients, would not benefit from multivitamins and minerals. Elderly people living in institutions such as nursing homes and homes for the elderly have in general a less optimal nutritional status as compared to their noninstitutionalized peers. Especially in institutions, pathogens can rapidly be transmitted from one person to another and the risk of complications is highest in institutionalized elderly persons. Although not evidence based with respect to respiratory infections, we would recommend these people to take multivitamin-mineral supplements. Some vitamin E in this supplement could be essential, but we have to be cautious about the dosage. Until the mechanism of vitamin E and its effect on infectious diseases has been clarified, we would not recommend people to take high dosages of vitamin E.

6.6 SUGGESTIONS FOR FUTURE RESEARCH

6.6.1 Study in institutionalized elderly people

It is still unresolved whether institutionalized elderly people will benefit from multivitamin-mineral supplementation in reducing acute respiratory infections. As mentioned before,

institutionalized elderly people are at high risk of infectious diseases and their complications and remain therefore an important target group. To overcome difficulties in the recruitment of relatively frail elderly people for future studies, as described previously in this chapter, several suggestions for an appropriate study design are presented. A fortified drink could be used as treatment, because a major reason for not willing to participate in our trial was that persons did not want to take capsules on top of their regular medication. The dosages of vitamins and minerals in this drink could be similar to the dosages as used in our capsules. Taken into account the probably higher prevalence of a suboptimal and deficient nutritional status, somewhat higher dosages might however be more adequate. Participation could be limited to people living in institutions, such as homes for the elderly and sheltered houses. Physical and mental situation of people living in these houses will in general be better as compared to persons living in nursing homes, while nutritional deficiencies are reported in both. If a trial will indeed be conducted in institutionalized elderly people, in-home nurses could supply the treatments and assess the infections. In that case, participants do not have to report the infections themselves in a diary. Because the spread of infections is generally higher in people living in institutions, resulting in a higher incidence, a lower number of participants may suffice to show an effect. Although the study of infectious diseases in frail elderly people will remain complicated, the major critical design issues might be resolved with a study design such as described above.

6.6.2 Mechanistic studies

We conducted the first trial that investigated the effect of a high dose of vitamin E on several parameters indicating illness-severity. We do not know what our effect on illness-severity means. We cannot exclude that our results represent a chance-finding, while the increased severity might even indicate an improved immune response. One might therefore question

whether an increased illness-severity is always unfavorable, especially if an improved immune response would translate into increased illness-severity.

If our results will be confirmed in future research, and vitamin E indeed exacerbates the severity, the question comes up by which mechanism vitamin E increases illness-severity. So far, several explanations have been presented for this phenomenon, such as a pro-oxidant action of vitamin E radicals when antioxidant networks are unbalanced, inhibition of glutathione S-transferase-pi and protein kinase C in macrophages, neutrophils, and monocytes.[57-61] Future studies could further explore the mechanism by which vitamin E may increase the severity of acute respiratory infections.

As described previously in this chapter, supplementation with alpha-tocopherol reduces concentrations of plasma gamma-tocopherol, as was also observed in our trial.[17-19] The reduced gamma-tocopherol concentrations might have resulted in the increased illness-severity because this isomer may have an anti-inflammatory activity. It was shown to inhibit cyclooxygenase activity in macrophages and epithelial cells, and binding to nitrogen oxide species which are produced during activation of phagocytes. However, its relevance for humans has not been investigated so far.[59;62;63] To overcome suppression of gamma-tocopherol, it may be fruitful to supply with a combination of both gamma- and alpha-tocopherol. First, the dosages of both alpha- and gamma-tocopherol and their proportion have to be defined in a dose-finding study. If dosages are well-defined and their effect on human immune response is clarified, a randomized controlled trial could address the effect of gamma- and alpha-tocopherol on acute respiratory infections.

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Appendix

Vitamin supplementation in elderly persons

Judith Graat, Evert Schouten, Frans Kok

JAMA 2003; 289: 173-174

To the editor: Ms Graat and colleagues[1] found that multivitamin supplements did not prevent acute respiratory tract infections in healthy elderly persons. The authors used a multivitamin and trace-element preparation that contained recommended daily allowances (RDAs) of vitamins but only 25% to 50% of the RDAs for minerals. The RDAs, however, are intended to prevent nutritional deficiency, and may be too low to produce further benefits.[2,3] Other studies, using nutrients in amounts based on physiological dose-response curves and immune responses, have found beneficial effects.[4,5]

I believe that most older individuals would benefit from the regular use of a multivitamin and mineral supplement that is based on evidence of dose-response curves rather than the theoretical RDAs.

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Financial Disclosure**:** Dr Chandra holds a US patent for a multinutrient preparation marketed as TALISMAN in two provinces in Canada.

1. Graat JM, Schouten EG, Kok FJ. Effect of daily vitamin E and multivitamin-mineral supplementation on acute respiratory tract infections in elderly persons: a randomized controlled trial. JAMA 2002; 288: 715-721.

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To the editor: In contrast to the results of Ms Graat and colleagues,[1] we found that mineral supplementation could reduce the incidence of infections in institutionalized elderly people.[2] Although we did not find significant reductions in the rate of acute respiratory infections in any group that received supplements, we found that patients who received a mineral (zinc plus selenium) supplement were more likely to have had no infections after two years ($p=0.06$). Moreover, in a subsample of 140 patients who were vaccinated against influenza, we noted a higher number of serologically protected patients after one and three months in the groups receiving mineral supplements ($p<0.05$). Finally, we observed that vitamin supplementation (containing vitamins E, ascorbic acid, and beta-carotene) seemed to have a negative effect on antibody titers following influenza vaccine ($p<0.05$).

Although Graat et al. found that vitamin E did not prevent respiratory infections in elderly persons, low doses of minerals such as zinc and selenium may help prevent respiratory infections, especially influenza.

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1. Graat JM, Schouten EG, Kok FJ. Effect of daily vitamin E and multivitamin-mineral supplementation on acute respiratory tract infections in elderly persons: a randomized controlled trial. JAMA 2002; 288: 715-721.

2. Girodon F, Galan P, Monget AL, et al for the MIN. VIT. AOX. Geriatric Network. Impact of trace elements and vitamin supplementation on immunity and infections in institutionalized elderly patients. Arch Intern Med 1999; 159: 748-754.

In Reply: We agree with Dr Chandra and Dr Girodon and colleagues that the composition of the multivitamin-mineral supplement may have contributed to our null-findings. We chose to use a commercially available supplement with only the micronutrients reported to be relevant to the immune response, and which are often suboptimal in the elderly population. For minerals, we included 50% of the RDA for elderly persons because of safety reasons: therapeutic and toxic levels of some minerals, such as copper, zinc, and iron, are reported to be close to another.[1]

It is unclear whether our micronutrient supplement would have resulted in beneficial effects on infectious diseases in an elderly population with optimal nutritional status, such as ours. Thus, we cannot rule out the possibility that higher levels of certain micronutrients might decrease the incidence of disease incidence. However, we did find evidence of serious adverse effects when vitamin E is supplied in amounts 17 to 20 times higher than the RDA.

We agree with Chandra that the optimal amounts of micronutrients adequate in improving immune response could be determined by performing dose-finding studies. Improvement in immune response, however, does not necessarily translate to a diminished frequency or severity of infections. Moreover, from a public health point of view, studying a clinical endpoint such as infectious diseases has much greater relevance.

The beneficial effects as observed by Chandra[2] and Girodon et al.[3] may be due to the higher proportion of patients in their samples with nutrient deficiencies. Moreover, the supplement group in Chandra's study had more nutrient deficiencies compared with placebo. The small sample size in the trial of Chandra is also a concern. Girodon et al.[3] do not describe the percentage of participants with suboptimal or deficient nutritional status among their institutionalized elderly patients. These persons are expected to be more frail and correspondingly have a less optimal nutritional status.[4]

It is possible that elderly individuals with suboptimal nutritional status may benefit from multivitamin-mineral supplementation at RDA level, or somewhat higher. However, potential adverse effects need to be monitored in their case.

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Summary

Thesis

‘Acute respiratory infections in elderly people: the role of micronutrients and lifestyle’

Introduction

Elderly people show an increased risk of acute respiratory infections, such as common cold, flu, and pneumonia, that are responsible for substantial morbidity and mortality in this age-group. A weakened immune function with increasing age and a suboptimal nutritional status are supposed to play a role. Since the elderly population is growing rapidly worldwide, considerable public health benefit may be obtained if changes in nutritional and lifestyle factors can reduce the risk of the infections. Our main objective was to study the effect of multivitamin-mineral and vitamin E supplementation on the incidence and severity of acute respiratory infections. Data of this randomized controlled trial in 652 elderly people were used to conduct observational studies on plasma carotenoid status and lifestyle, i.e. alcohol consumption, smoking, and physical activity in relation to the infections. To gain insight in the causative viruses, virological assessment was performed in a subset of persons with and without symptoms of acute respiratory infection. Results of this analysis were also used to evaluate the quality of the subjective self-reports about the presence or absence of infection.

Virological assessment

Chapter 2 presents the results of a one-year prospective virological assessment during 107 case-episodes of elderly people with symptoms of acute respiratory infection and during 91 control periods of elderly people without such symptoms. Polymerase chain reaction and serology were used to check for the nine most common respiratory viruses, including *Mycoplasma pneumonia*. Fifty-eight percent of the self-reported infections was laboratory-confirmed, with rhinoviruses being the most common pathogens (32%), followed by coronaviruses (17%), and influenzaviruses (7%). In four percent of persons without symptoms of acute respiratory infection a virus was demonstrated. These subclinical infections were caused by rhinoviruses (2%) and coronaviruses (2%). Thus, these data suggest that rhinovirus

infections are responsible for substantial morbidity in elderly people. Besides, subclinical respiratory infections occur occasionally, but still in four percent of persons without symptoms. This group may be an unrecognized source of infection and transmit the pathogen to others.

Vitamin E and multivitamin-mineral supplementation

In chapter 3, we investigated the effect of 200mg of vitamin E and multivitamins-minerals in doses near the recommended dietary allowance on acute respiratory infections. This 15-months randomized controlled trial comprised 652 well-nourished, relatively healthy elderly people (aged 60-95 years). Presence or absence of infections was daily self-reported in a diary. The self-report was validated by nurse telephone contact, home-visits, rectal temperature, and microbiological and serological testing in a subset of persons. Participants had on average 1.6 acute respiratory infections per person per year, whereas 32% did not experience an infection during the observation period. Both vitamin E and multivitamins-minerals did not have beneficial effects on the incidence and severity of the infections. We demonstrated a non-significant increased risk of twelve percent in groups receiving vitamin E and a non-significant reduced risk of five percent in groups receiving multivitamins-minerals. Instead, we observed significant adverse effects of vitamin E on illness-severity. Illness-duration was 19 vs 14 days; no. of symptoms was six vs four; presence of fever was 37% vs 25%; and presence of activity-restriction was 52% vs 41% in the vitamin E group compared to the no-vitamin E group. Healthy well-nourished elderly people will therefore not benefit from vitamin E and multivitamin-mineral supplementation to reduce the risk of acute respiratory infections. If the unfavorable effects on illness-severity will be confirmed, it is advisable to be cautious about taking vitamin E supplements.

Carotenoids

In chapter 4, we evaluated the relation of plasma status of six major carotenoids, i.e. beta-carotene, alpha-carotene, beta-cryptoxanthin, lycopene, lutein, and zeaxanthin with the incidence and severity of acute respiratory infections. Baseline data of the intervention trial were used to divide plasma carotenoid concentrations into carotenoid categories. Frequency and severity of acute respiratory infections during the previous year were reported in a questionnaire. A significant reduced risk of the infections of 29% was observed at high and of 26% at intermediate plasma beta-carotene status, whereas beta-carotene status was not related to illness-severity. No association was observed for the other carotenoids investigated. Thus, a high plasma beta-carotene status may reduce the incidence of acute respiratory infections, whereas other main carotenoids may not.

Lifestyle

In chapter 5, we studied the relation of lifestyle factors, i.e. alcohol consumption, smoking, and physical activity with the incidence and severity of acute respiratory infections. Data were used from the trial, which is described previously. Lifestyle factors were assessed at baseline by means of validated questionnaires. Self-reported infections were assessed by nurse telephone contact, home visits, rectal temperature, and microbiology and serology testing in a subset of participants. Moderate/heavy alcohol drinking (men >3 drinks/day and women >2 drinks/day) showed a significant 33% increased risk, light drinking (men 1<glasses/day≤3 and women 1<glasses/day≤2) a non-significant 22% increased risk, and occasional drinking (0<drinks/day≤1) a significant 31% increased risk of the infections, compared with abstainers. Alcohol consumption was not related to illness-severity. Smoking and physical activity were not related to the infections. All results were adjusted for age and sex and remained unaltered after additional adjustment for lifestyle factors and other potential confounders. Thus, of the

lifestyle factors we investigated, only alcohol may increase the risk of acute respiratory infections in relatively healthy well-nourished elderly people.

Discussion and concluding remarks

In chapter 6, we have put our studies into perspective by addressing several methodological aspects. The relatively low dosages in our multivitamin-mineral supplement might have played a role in our null-findings, although we suppose that the well-nourished status of the population is of more importance. So far, only our trial investigated the effect of vitamin E on illness-severity, showing results contradictory to our hypothesis. Although our results were statistically significant, we can not exclude that our unfavorable findings were due to chance. The limited information on the mechanism of vitamin E on respiratory infections hampers the definition of the optimal dosage of this vitamin. So far, we see no reason to recommend elderly people, especially those who are already well-nourished, to take multivitamin-mineral or vitamin E supplements. Future studies could address the mechanism of vitamin E with respect to respiratory infections. Besides, it is worthwhile to investigate whether elderly people with a suboptimal nutritional status will benefit from multivitamin-mineral supplementation. Our observational analyses were sensitive to selection and information bias. In the analysis on carotenoids, the self-report of acute respiratory infections during the previous year remains a critical issue. Besides, selection bias might explain our beneficial finding of beta-carotene. Our results indicating that a high plasma beta-carotene status is associated with a reduced frequency of acute respiratory infections, should therefore have to be interpreted with caution. In the analysis on lifestyle factors, we cannot rule out that selection and information bias have influenced our results. Therefore, it is not warranted to recommend elderly people to change their habits of life, such as alcohol consumption, smoking, and physical activity in order to reduce the risk of acute respiratory infections.

Samenvatting

Proefschrift

‘Acute luchtweginfecties bij ouderen:

de rol van micronutriënten en leefstijl’

Inleiding

Acute luchtweginfecties is een verzamelnaam voor onder meer neusverkoudheid, keelpijn, griep en longontsteking. Verschillende virussen kunnen deze klachten teweegbrengen en uit het klachtenpatroon valt niet af te leiden wat het veroorzakende luchtwegvirus is. Ouderen hebben een verhoogd risico op zulke acute luchtweginfecties en de daarmee gepaard gaande complicaties. Gemiddeld hebben ouderen één tot twee acute luchtweginfecties per jaar. De infecties kunnen een ernstig ziekteverloop en sterfte bij deze bevolkingsgroep tot gevolg hebben. Zo sterven er jaarlijks in Nederland ongeveer 2.000 ouderen aan griep. Naarmate mensen ouder worden gaat het immuunsysteem (afweer) geleidelijk minder goed functioneren. Een minder goed functionerend immuunsysteem, alsmede een minder goede voedingsstatus, zouden een belangrijke rol kunnen spelen bij het verhoogde risico op infectieziekten.

Het aantal ouderen neemt wereldwijd sterk toe. Als gunstige veranderingen in de voedingsstatus of leefstijl het risico op acute luchtweginfecties zouden kunnen verminderen, zou er een aanzienlijk volksgezondheidsvoordeel behaald kunnen worden. De voedingsstatus van ouderen kan bijvoorbeeld verbeterd worden door extra micronutriënten zoals vitaminen en mineralen te geven. Veranderingen in leefstijl kunnen betrekking hebben op alcohol gebruik, roken of lichamelijke activiteit.

Het belangrijkste doel was te onderzoeken of het geven van multivitaminen-mineralen en/of vitamine E het aantal en de ernst van luchtweginfecties verminderde. Om dit te onderzoeken hebben we een groot onderzoek uitgevoerd waaraan 652 ouderen mee hebben gedaan. De gegevens van dit onderzoek hebben we ook gebruikt om andere vraagstellingen te onderzoeken, namelijk of carotenoïden (stoffen met name voorkomend in groente en fruit) en leefstijl factoren als alcohol gebruik, roken en lichamelijke activiteit verband houden met

luchtweginfecties. Om inzicht te krijgen in de virussen die de meeste acute luchtweginfecties veroorzaken, hebben we testen op aanwezigheid van luchtwegvirussen gedaan bij mensen met en zonder klachten van infectie.

Testen op luchtwegvirussen

In hoofdstuk 2 zijn de resultaten gepresenteerd van een één jaar durende studie waarin getest werd op de aanwezigheid van de negen meest voorkomende luchtwegvirussen. Alle 652 ouderen hielden elke dag in een dagboekje bij of ze wel of geen last hadden van luchtwegklachten. Uit deze groep deelnemers hebben we een steekproef getrokken van 107 ouderen met pas begonnen verkoudheidsklachten en van 91 controle personen die op dat moment geen verkoudheidsklachten hadden. Bij zowel de mensen met verkoudheidsklachten (aangeduid met ‘patiënten’) als bij de mensen zonder klachten (aangeduid als ‘controles’) is een uitstrijkje gemaakt van neus- en keelslijm en is bloed afgenomen binnen drie dagen nadat de klachten begonnen bij de patiënt. Na twee tot vier weken is er opnieuw bloed afgenomen bij zowel de patiënt als de controle. De laboratorium methode ‘polymerase chain reaction’ is gebruikt om in de neus-keel uitstrijkjes virusmateriaal aan te tonen. Serologische bepalingen zijn gedaan om in het bloed antistoffen tegen virussen aan te tonen.

Het bleek dat bij 58% van de patiënten een luchtwegvirus aangetoond kon worden. De meest voorkomende virussen waren rhinovirussen (32%), coronavirussen (17%) en influenzavirussen (7%). Ook in vier procent van de controles werd een virus aangetoond: twee procent rhinovirussen en twee procent coronavirussen. Onze resultaten laten zien dat rhinovirussen vaak de veroorzakers zijn van acute luchtweginfecties bij ouderen en dat ook subklinische infecties kunnen voorkomen. Mensen met een subklinische infectie - personen die wel een virus bij zich dragen, maar geen verkoudheidsklachten hebben - kunnen een virus ongemerkt verspreiden naar anderen.

Multivitaminen-mineralen en vitamine E

In hoofdstuk 3 is het hoofdonderzoek gepresenteerd over vitamine supplementen. Aan dit onderzoek deden 652 ouderen mee van 60 tot 95 jaar, 325 mannen en 327 vrouwen. De quetelet-index, een maat die het gewicht t.o.v. de lengte aangeeft, gaf aan dat het om goed gevoede ouderen ging. Ook had bij aanvang van het onderzoek slechts zes procent van de deelnemers verminderde concentraties van vitamin C in het bloed en 1.3 procent van vitamine E. Twee procent van de deelnemers woonde in verzorgingshuizen of aanleunwoningen. De groep deelnemers wordt daarom beschouwd als niet-geïnstitutionaliseerd.

De 652 ouderen kregen gedurende 15 maanden elke dag een supplement te slikken bestaande uit één van de vier volgende samenstellingen: (1) een hoge dosis vitamine E (200mg, ongeveer 17-20 maal de hoeveelheid die in uw dagelijkse voeding hoort te zitten); (2) allerlei vitaminen en mineralen (in ongeveer een hoeveelheid zoals die in uw voeding hoort te zitten); (3) zowel vitamine E als multivitaminen-mineralen; of (4) placebo, oftewel ‘neppil’. Naast het dagelijks slikken van de voedingssupplementen hielden alle ouderen ook een dagboekje bij waarin ze elke dag noteerden of ze wel of geen last hadden van luchtwegklachten. Indien de deelnemers luchtwegklachten hadden, meldden ze dit telefonisch bij het onderzoeksteam, dat vervolgens controleerde of de klachten inderdaad een luchtweginfectie betroffen. Bij een steekproef van mensen werd vervolgens een huisbezoek verricht om de klachten te verifiëren, bloed af te nemen en een neus-keel uitstrijkje te maken om te checken op aanwezigheid van luchtwegvirussen (zie hoofdstuk 2). Koorts is ook een indicator van infectie. Alle deelnemers kregen daarom een thermometer om op dagen met klachten hun lichaamstemperatuur te meten. De ouderen hadden tijdens het onderzoek gemiddeld 1.6 acute luchtweginfecties per persoon per jaar. In totaal had 32% van de deelnemers geen enkele luchtweginfectie gedurende de tijd dat ze meededen aan het onderzoek. Zowel vitamine E als multivitaminen-mineralen hadden geen gunstig effect op het aantal luchtweginfecties en de ernst van de

infecties. De ouderen die vitamine E slikten, hadden een twaalf procent hoger risico om een luchtweginfectie te krijgen. Dit was niet statistisch significant, wat wil zeggen dat het een toevalsbevinding zou kunnen zijn. De ouderen die multivitaminen-mineralen kregen, hadden een niet statistisch significant vijf procent lager risico. Daarentegen zagen we een statistisch significante verhoging van de ernst in de vitamine E groep. Wanneer we de groep die vitamine E kreeg vergeleken met de groep die dat niet kreeg, was de ziekteduur 19 versus 14 dagen, het aantal symptomen zes versus vier, het percentage mensen met koorts 37% versus 25%, en het percentage mensen met restrictie van activiteit (bijv. in bed blijven) 52% versus 41%. Onze conclusie was daarom dat vitamine E en multivitaminen-mineralen het risico op acute luchtweginfecties niet verminderen bij gezonde, goed gevoede ouderen. We willen zulke ouderen dan ook niet aanraden deze supplementen te nemen om luchtweginfecties te voorkomen. Als onze ongunstige resultaten van vitamine E bevestigd zullen worden in toekomstig onderzoek, moet men daarentegen voorzichtig zijn met het nemen van vitamine E supplementen in hoge doseringen.

Carotenoïden

Carotenoïden zijn stoffen die met name in groente en fruit voorkomen en daarin zorgen voor de bijvoorbeeld rode, groene, gele, of oranje kleur. Mensen kunnen deze stoffen niet zelf aanmaken en groente en fruit zijn daarom de belangrijkste voedingsbronnen van carotenoïden. In hoofdstuk 4 zijn de resultaten gepresenteerd van een onderzoek over de relatie tussen de zes meest voorkomende carotenoïden in het bloed en het optreden van luchtweginfecties. Het bloed en andere gegevens van de 652 ouderen die meededen aan het vitamine suppletie onderzoek (zie hoofdstuk 3) werden voor dit onderzoek gebruikt. Gegevens over het aantal en de ernst van acute luchtweginfecties in het voorafgaande jaar werden verkregen door alle deelnemers een vragenlijst in te laten vullen. In deze vragenlijst werd gevraagd hoe vaak de

deelnemers een verkoudheid, griep, keelpijn, voorhoofdsholte ontsteking en longontsteking hadden gedurende het voorafgaande jaar. Vervolgens werd voor iedere infectie de ernst ingevuld: koorts, in bed gebleven, huisarts bezoek en gebruik van medicijnen. We zagen een statistisch significant 29% lager risico om een luchtweginfectie te krijgen bij de mensen die een hoge concentratie beta-caroteen in hun bloed hadden. Mensen met een gemiddelde concentratie beta-caroteen, hadden een 26% lager risico t.o.v. de mensen met een lage concentratie in hun bloed. Beta-caroteen was niet gerelateerd aan de ernst van acute luchtweginfecties. De overige vijf carotenoïden die we onderzochten hadden geen relatie met de frequentie noch met de ernst van de infecties.

Onze conclusie was daarom dat een hoge beta-caroteen concentratie in het bloed het risico op het krijgen van het acute luchtweginfectie vermindert, terwijl andere veel voorkomende carotenoïden dat niet doen.

Leefstijl

In hoofdstuk 5 zijn de resultaten gepresenteerd van een onderzoek naar leefstijl en acute luchtweginfecties. Gegevens van het vitamine suppletie onderzoek (zie hoofdstuk 3) werden gebruikt om dit te onderzoeken. Leefstijl factoren - alcohol gebruik, roken en lichamelijke activiteit - werden bij de start van het onderzoek vastgesteld met behulp van een vragenlijst. Onze resultaten laten zien dat ouderen die alcohol drinken een ongeveer 30% hoger risico hebben om een acute luchtweginfectie te krijgen, dan ouderen die geen alcohol drinken. Alcohol consumptie was niet gerelateerd aan de ernst van de acute luchtweginfecties. De twee andere leefstijl factoren die we onderzochten - roken en lichamelijke activiteit - waren niet gerelateerd aan de infecties. Dus van de leefstijl factoren die wij gemeten hebben, vonden wij alleen aanwijzigingen voor een mogelijk effect van alcohol consumptie op acute

luchtweginfecties bij ouderen. Of dit werkelijk een oorzaak-gevolg relatie is, is niet met zekerheid te zeggen.

Discussie en conclusies

In hoofdstuk 6 hebben we de hierboven beschreven onderzoeken bediscussïeerd met betrekking tot de opzet (methodologie).

De relatief lage doseringen van de vitamines en mineralen in ons multivitaminen-mineralen supplement zouden een rol gespeeld kunnen hebben in het feit dat wij hiervan geen gunstig effect vonden. Echter, wij vermoeden dat de goede voedingstoestand van de ouderen in ons onderzoek mogelijk ook een verklaring zou kunnen zijn.

Tot nu toe zijn wij de enigen die het effect van vitamine E op de ernst van luchtweginfecties onderzocht hebben. Onze resultaten zijn tegengesteld aan wat we vooraf verwacht hadden. Ondanks dat onze resultaten zeer waarschijnlijk niet op toeval berusten, mogen we een kleine resterende kans dat dit toch zo is niet uitsluiten. De geringe kennis over het mechanisme van een effect van vitamine E op infectieziekten maakt het bepalen van de meest optimale dosis van vitamine E onmogelijk. We concluderen dat we tot nu toe geen reden zien om ouderen, met name de ouderen die goed gevoed zijn, te adviseren om multivitaminen-mineralen te nemen om acute luchtweginfecties te voorkomen. Indien onze resultaten over vitamine E bevestigd worden in ander onderzoek, moet men zelfs voorzichtig zijn met het nemen van hoge doseringen vitamine E. Het is interessant om te kijken of ouderen met een minder goede voedingstoestand wel baat hebben bij multivitaminen-mineralen. Daarnaast kunnen toekomstige onderzoeken zich richten tot het mechanisme van vitamine E met betrekking tot acute luchtweginfecties.

De onderzoeken over carotenoïden en leefstijl waren observationeel van karakter en we moeten daarom voorzichtig zijn bij de interpretatie van de resultaten. In het onderzoek over

carotenoïden was bijvoorbeeld de herinnering van infecties gedurende het voorafgaande jaar een kritiek punt. Daarnaast kan het zijn dat er andere belangrijke verschillen zijn tussen de mensen met hoge en lage carotenoïden concentraties in het bloed, die het gevonden verband kunnen verklaren. Mensen met lage carotenoïden concentraties zouden bijvoorbeeld een iets slechtere gezondheid kunnen hebben en zodoende een verhoogd risico op luchtweginfecties. In het onderzoek over leefstijl factoren, kunnen we niet uitsluiten dat ook hier de groepen niet goed vergelijkbaar waren. Andere leefstijlaspecten van mensen met weinig lichamelijke activiteit of mensen die nooit alcohol drinken kunnen verschillen van die van mensen met veel lichamelijke activiteit of mensen die wel alcohol drinken. Daarnaast kan het zijn dat bijvoorbeeld rokers hun luchtwegklachten anders gerapporteerd hebben, dan dat niet-rokers dat deden. Ook bij dit onderzoek moeten onze resultaten daarom met enige voorzichtigheid geïnterpreteerd worden. We zien daarom vooralsnog geen reden om ouderen te adviseren hun alcohol gebruik, rookgedrag en lichamelijke activiteit te veranderen met het doel acute luchtweginfecties te voorkomen.

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About the author

Judith Marie-Anne Graat was born in Helmond, The Netherlands on the 14th of April in 1974. In the summer of 1993, she completed secondary school (VWO) at the 'Kruisheren Kollege' in Uden before she started her study Biological Health Science at the University of Maastricht, The Netherlands. As part of this study, she started her first research project in November 1996 at the division of Human Biology of the University of Maastricht on energy expenditure and fat metabolism in obese individuals. Her curiosity for micronutrients brought her to conduct her second research project at the division of Human Nutrition of the Wageningen University, The Netherlands. From September 1997 until May 1998, she conducted an intervention trial in rural

Indonesian infants on zinc supplementation and intestinal permeability. From May until October 1998 the division of Human Nutrition appointed her as a research associate to carry out research on sensory specific satiety. In September 1998, she obtained her MSc degree in Biological Health Science. In October 1998, she was appointed as a PhD-fellow to conduct a trial on micronutrient supplementation and acute respiratory infections, as described in this thesis. She joined several conferences and followed several courses on nutrition, epidemiology, and management within the framework of the educational program of the Graduate School VLAG (Food Technology, Agrobiotechnology, Nutrition and Health Sciences). In July 1999, she attended the Annual New England Epidemiology Summer Program at Tufts University, Boston, USA. During the October meeting of the nutrition working group of the Dutch organization for scientific research (NWO), she won the ‘Young Investigator’s Award – Foppe ten Hoor’ for her intervention trial as described in this thesis. In March 2002, she was selected to participate in the European Nutrition Leadership Program in Luxembourg. Since October 2003, she works as a product design technologist at Masterfoods, Veghel, The Netherlands.

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