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Impact of nutritional supplements on health indices in elderly people

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ABSTRACT

Impact of nutritional supplements on health indices in elderly people.

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Life expectancy in both the Western and non-western population has been increasing over the past centennial, due to improved hygiene, the discovery of medicines such as antibiotics and economic welfare. The consequence for society of this ageing of our population is an increased need for medical and social care and thus a burden of costs for health care. At the level of individual people an increased life expectancy is appealing, but only if accompanied by a preservation of a certain health status. Low intakes or plasma levels of nutrients have been related to several areas of physiological decline often observed in elderly people.
This thesis describes the effects of provision of a complete nutritional supplement to elderly who are at risk for impaired nutritional status. Two studies in the population of psychogeriatric nursing home residents demonstrated that both short and longer-term use of a nutritional supplement was well accepted and tolerated in this target group. Furthermore, body weight and plasma levels of micronutrients increased upon supplementation.
The results of a 6-months randomised placebo controlled intervention study in elderly living in homes for the elderly or sheltered housing are also reported. The effect of a nutritional supplement (containing macro- and micronutrients) or a non-energetic placebo on health indices was evaluated. The group receiving the supplement had significant improvements in plasma vitamin levels, weight, sleep, immune function, antioxidant levels and cognitive function. The objective for subjects to maintain intake from the regular diet was reached, and thus their total nutrient intake increased when using the supplement.
In conclusion, the provision of a nutritional supplement can improve several health indices in elderly people who are at risk of nutritional deficiencies. Therefore, the strategy to improve health of elderly people should incorporate the option of providing nutritional supplements.

CONTENTS

1

General introduction

Life expectancy in both the Western and non-western population has been increasing over the past centennial, due to improved hygiene, the discovery of medicines, such as antibiotics, and economic welfare. In the Netherlands, 75% of all deaths among men and 85% of all deaths among women occur after the age of 65[1]. Not only has survival per se increased, also the life expectancy of people has been increasing from 70.7 years for men and 76.9 for women in 1990 to 71.9 for men and 78.2 for women in 2000[1]. The consequence of this increased life expectancy, and the relatively decreased birth rate, is that the Dutch population currently contains 13,6 % elderly people (defined as people over 65)[1]. This number is expected to increase over the coming years (see figure 1).

Figure 1. *Demographic scheme of age distribution of the Dutch population from 1900 to 2050[1].*

The consequence for society of this ageing of our population is an increased need for medical and social care and thus a burden of costs for health care. An example of increasing costs is the higher demand for care of elderly living in elderly homes in the Netherlands over the past years as demonstrated in figure 2. As a consequence, a large proportion of the health care costs in the Netherlands is attributed to people over 65 years of age[2].

Figure 2. *Need for care in elderly homes in the Netherlands[1].*

At the level of individual people an increased life expectancy is appealing, but only if accompanied by a preservation of a certain level of health. As the physiological reserves in elderly people decline, they are at high risk for events with a large impact on quality of life[3]. Furthermore, an increased need for care at old age is a heavy burden to relatives[4]. Due to the increased life expectancy this can be necessary for a long time and thus children are caring for their parents until they are in old age themselves.
The obvious aim is thus to keep this growing segment of the population in good health.

Nutrition is one of the factors that play an important role in maintaining health in elderly people. Poor nutritional status (as defined by serum albumin and body weight) has been related to both morbidity and mortality[5,6]. Low intakes or plasma levels of nutrients have been related to several areas of physiological decline often observed in elderly people. Among these relations are bone density[7], memory[8], cardiovascular disease[9], physical functioning[10], immune function[11], lung function[12] and eye function[13].

The term elderly people is a broad description for a population group. In general, the definition of elderly people is having an age over 60 or 65 years. However, beyond this age it is difficult to differentiate by age within the group of elderly people. A person aged 65 may already be severely functionally impaired, whereas a person aged 80 may still be fully functional. Several ways to categorise elderly people have been used. These either depend on functional status (frail vs. non-frail[14] or successful agers vs. accelerated agers[15,16]), health status (apparently healthy vs. not healthy[17] or hospitalised vs. not hospitalised), living location (institutionalised vs. free-living[18]), among which a distinction can be made between hospitals, nursing homes (psychogeriatric and somatic), homes for the elderly, sheltered housing) or need for care (hospital, nursing home, meals on wheels clients[19]).

Inadequate intake of one or more nutrients has been reported in 24% of elderly men and 47% of elderly women[20]. Furthermore biochemical deficiencies are often reported in elderly people[21]. In general, the causes of these deficiencies are threefold: 1) inadequate intake which might be caused by reduced macronutrient requirement, poor dietary habits, isolation, depression, dental or chewing problems, medication or dementia, 2) increased nutritional requirements due to fever, infection or dementia and 3) losses of nutrients or reduced absorption, for instance protein loss from wounds, glycosuria, diarrhoea or medication[22,23].

The group of elderly that has mostly been reported to have an impaired nutritional status is institutionalised elderly. In the Netherlands, 3% of elderly people aged 65-79 are living in institutions, and this number increases to 21% for age 80 years and over[1]. The nutrient intake of Dutch institutionalised elderly has been investigated previously and vitamin deficiencies are prevalent, especially for the vitamins B1, B6, C and D[24].

However, also elderly living at home may be at risk for nutritional deficiencies. Also in this group nutrient intake is often inadequate[25,26] and plasma levels of vitamin D, vitamin B6

and vitamin B12 are deficient in a large proportion[21].

There are many different ways to improve nutritional status, mostly aimed at improving nutrient intake. An improvement of quantity of intake, quality of intake or a combination of both may be established. Intake quantity may be increased by improving access to food (e.g. meals on wheels programs), feeding assistance[27], improving meal ambience[28], flavour of the food[29,30], or exercise[31]. Quality of intake may be improved by dietetic counselling, provision of nutrient dense foods[32], or nutritional supplementation (by e.g. drinks or tablets). Nutritional supplementation results in improvements in both quantity and quality of intake and therefore affects nutritional status. Furthermore, it has been shown that in malnourished subjects, weight gain has a positive impact on both morbidity and mortality[6].

When choosing for nutritional supplementation, different aspects of a supplement should be taken into account depending on the nutritional status of the elderly person. Either a specific nutrient is to be supplied (e.g. protein, or a vitamin) or a broad range of nutrients (e.g. micronutrients or macro- and micronutrients). The timing of supply is either before occurrence of any signs or consequences of deficiencies (e.g. primary preventive; low intake: energy, calcium supplements), upon first signs of deficiencies (e.g. secondary preventive; weight loss, low plasma vitamin D) or when (deficiency) disease has already occurred (e.g. curative; illness, osteoporosis). Duration of supplementation is either long term, or short term in an at risk situation with discontinuation when this risk situation has resolved. Dosage of nutrients is dependent on duration, a choice may be made for a maintenance dose to maintain optimal nutrient status, or a high dose booster to improve nutritional status in a very short term.
In any case, the nutritional supplement provided should be condition-specific: it should contain all the nutrients required in a specific situation to establish optimal effects.

To date, our knowledge about the effects of supplementation with a combination of macro- and micronutrients on functional outcomes in elderly who are at risk of poor nutritional status is limited. Most studies so far have investigated only nutritional status as outcome parameter or evaluated effects of single nutrients or single functional parameters. Taking into account all these factors, this thesis has taken nutritional related health indicators as outcome measures, which are discussed below (p.17).

Intervention and design

There is a diversity of nutrients that may have an impact on health in elderly people[9], therefore a complete, nutrient dense nutritional supplement was designed. The levels of micronutrients selected were physiological, around 100% of recommended daily allowances (RDA) depending on safety and processing limitations. Because studies with antioxidants have often been performed with higher doses (e.g. up to 20x RDA), and this may be required for these nutrients to exert optimal antioxidant function[33-35] those were supplied in higher amounts. The selected levels were considered to be still in the physiological range (up to 5x RDA). Since not only micronutrients but also energy is important to maintain physical function, an energy containing drink was provided.

Due to physiological changes in elderly people, like increased satiety and decreased hunger[36], the supplement was administered in such a way that habitual food intake would be influenced as little as possible. Therefore, the volume of the supplement was relatively low (125 ml), requiring administration twice daily to attain an increase in energy intake of about 15 % of the regular daily energy intake. The supplement was to be taken between meals in order not to influence the consumption of regular meals[37].

To control as much of the study as possible, a placebo was incorporated in the study design, to account for any placebo effects occurring by merely providing a supplement. To improve compliance and prevent dropouts, an investigator visited all subjects on a regular basis (every 2 weeks).

An intervention period of 6 months was selected, as this is probably a maximally feasible intervention time to assure compliance and a sufficient time for changes in functional parameters. The basis of this was to allow for a sufficient time for the elderly subjects to functionally adapt to their improved nutritional status. In some studies lack of measurable effects has been reported for interventions of 12 weeks[38].

To evaluate the effect of such nutritional supplementation different target groups were selected.

In our first study (Chapter 2) psychogeriatric nursing homes residents were selected, because in this group weight loss is a very common problem and the incidence of illness is relatively high.

To evaluate the feasibility of longer term supplementation and its biochemical effects a pilot study was performed (Chapter 3). Also psychogeriatric nursing home residents were selected, as the risk of malnutrition has been reported to be notably higher in elderly

patients who live in nursing homes than those living at home[39].

Since positive effects of supplementation on body weight and plasma vitamins in psychogeriatric nursing home residents with impaired nutritional status were found, it was the intention to extend the results to a larger group of elderly people with impaired nutritional status. Therefore, for the subsequent study reported in Chapter 4-8, a group of frail elderly people was selected, who were living in a semi-institutionalised setting (homes for the elderly or sheltered housing) and had a relatively low body mass index (≤ 25 kg/m^2). When calculating from the fact that the population of homes for the elderly in the Netherlands is 105.000 persons[1] and 50% of all elderly has a BMI below 25 kg/m^2 [1], this group is thus representative of about 50.000 elderly people in the Netherlands. However, also a large number of elderly people who are not institutionalised would fall within this group because they require care at home.

To evaluate the effects of provision of nutritional supplements to different groups of elderly people a range of outcome parameters was selected. These were firstly based on the evaluation of (changes in) nutritional status of the study subjects.

Energy and nutrient intake

The prevalence of inadequate micronutrient intakes gradually decreases with higher energy intakes, but of elderly people with energy intakes above 1500 kcal, still 19% of men and 26% of women had inadequate intake of at least one micronutrient[20]. In order to evaluate the nutritional status of a population an estimation of intake of energy and micronutrients needs to be made. Furthermore, it is necessary to know whether the supplement is consumed besides a normal regular food intake. It is possible that displacement due to the nutritional intervention occurs, which might mask any effects of the intervention.

Biochemical parameters of nutrition and health status

Besides nutrient intake, plasma levels give a closer indication of nutritional status of the study group. Furthermore, it is expected that plasma levels of nutrients provided in a supplement rise upon supplementation[40]. Lack of such rise could indicate inadequate compliance

Homocysteine is used as an indicator of nutritional status of B-vitamins as its levels are determined by intake of predominantly folate, vitamin B12 and vitamin B6[41-43]. High levels

of homocysteine have been related to increased risk for dementia, heart disease and even death[44]. Intervention studies with B-vitamins have shown that a lowering of plasma homocysteine levels can be obtained[45].

The free radical theory of ageing implicates a role for oxidative stress in the ageing process and the occurrence of several diseases[46]. Low intake of antioxidant nutrients such as vitamin E and vitamin C have been related to the risk of pathologies[47-50] and intervention with antioxidant vitamins has shown positive effects[51].

Body composition

Low body weight has been related to an increased risk of hospitalisation[52]. Furthermore, in elderly people a selective loss of fat free mass over fat mass occurs, leading to less muscle mass available for physical activity[53]. To evaluate whether provision of a supplement leads to a weight gain that is related to an increase not only in fat mass (which cannot serve as a source of functional improvement) but also in fat free mass, the quality of the weight gain needs to be assessed[54].

Secondly, we made a selection of functional outcome parameters of which impairment in the elderly and their relation to impaired nutritional status and health have been reported.

Physical functioning

Besides the effect of ageing per se, malnutrition in elderly people is also cause of a reduction in muscle strength and functional decline such as reduced walking time[55]. Functional changes due to supplementation can be evaluated by outcome measures such as grip strength[40,56], quality of life questionnaires[57,58] and activities of daily living questionnaires[40,59].

Cognitive function

Cognitive function has been shown to decrease with ageing[60]. Especially the area of declarative memory, a domain of memory associated with conscious recollection and usually assessed with recall or recognition tests, is consistently impaired in elderly people[61]. There are indications that impaired status of B-vitamins or antioxidants may decrease cognitive functioning[8] and supplementation with those will lead to an increase of cognitive functioning[62-65]. Several tests are available to measure cognitive function in the domains that have been identified to decline with ageing and these are used for evaluation of

nutritional intervention.

Immune function

Immune function in general declines with ageing[66,67]. Currently many elderly people are vaccinated for influenza infection (70%)[1], but due to the decline of immune response concern is raised that they are unable to reach titre levels for full protection. Malnutrition of both protein-energy and micronutrients has been related to impaired immune function[11] and reversal of the malnutrition can improve immune function[68-71]. To evaluate immune function *ex vivo* (the activity of T-cells)[69] as well as *in vivo* (response to vaccination)[70] methods are used.

Objective and outline of the thesis

The aim of the studies described in this thesis was to evaluate the effects of a nutrient dense nutritional supplement on health in elderly people, with emphasis on functional endpoints such as cognition, immunity and quality of life.

In Chapter 2 the short-term effect of provision of a nutritional supplement immediately after onset of infectious disease on body weight and other anthropometric parameters is described.

Chapter 3 explores the effects of provision of a nutrient dense nutritional supplement on body weight and plasma vitamin status in psychogeriatric nursing home residents.

Chapter 4 is the first chapter describing a broadened study of Chapter 3. In this chapter, effects of supplementation on body composition, physical functioning and quality of life in elderly living in elderly homes or sheltered housing are reported.

Chapter 5 explores the effects of the intervention on a range of tests of cognitive function and plasma levels of homocysteine and vitamin B12.

In Chapter 6 a biochemical evaluation of antioxidant status and the effect of supplementation is made.

Chapter 7 reports the effect of the intervention on immune function, as measured by response of isolated immune cells to a stimulant.

Chapter 8 includes a substudy in subjects who received influenza vaccination at the end of the study. The effect of nutritional supplementation on the efficacy of the vaccination was evaluated.

In Chapter 9 the results from Chapters 2 to 8 are summarised and methodological issues in this type of nutritional intervention are discussed.

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2

Early supplementation after infection in nursing home residents.

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Submitted

Abstract

Background: Many elderly with Alzheimer's disease experience weight loss. Illness and inadequate regain after a disease period are considered as contributory causes of progressive weight loss in psychogeriatric patients. In the present study we investigated whether early use of a liquid nutrition supplement immediately after onset of acute illness from infection can prevent weight loss in elderly psychogeriatric nursing home residents.

Methods: Randomized controlled trial of 5 weeks after the onset of illness. Thirty-nine psychogeriatric nursing home residents (aged ≥ 65 y) were randomized, 34 residents completed the study period. A liquid nutrition supplement (200 ml) once daily immediately after diagnosis of infection or standard treatment (enriched food after referral to a dietitian for weight loss). Body weight, mid upper arm circumference, calf circumference, triceps skinfold thickness, dietary energy intake and need for care were measured at start and 5 weeks after onset of illness.

Results: Weight change during the study period was significantly different between standard (-0.4 kg) and supplement (+0.8 kg) group (p=0.040). No significant differences were observed in changes of mid upper arm circumference, triceps skinfold thickness, calf circumference and energy intake between groups. In both groups the need for care similarly increased during the disease period.

Conclusions: We conclude that early provision of a liquid nutrition supplement immediately after onset of acute illness from infection increases weight in elderly psychogeriatric nursing home residents.

Introduction

Many elderly people with Alzheimer's disease (AD) experience weight loss[1] which is a predictor of mortality in these AD patients[2]. Illness and inadequate restoration of body weight after a disease period have been mentioned as contributory causes of the observed weight loss in psychogeriatric residents[3]. In concert with other risk factors they may induce progressive weight loss via a vicious circle (higher susceptibility to disease, leading to disease and subsequent weight loss)[4]. Prevention of weight loss during illness is therefore considered to be very important.

The type of illness resulting in weight loss is either chronic or acute. The most common acute illness in the elderly is infection[5]. In particular upper airway infections and urinary tract infections have been related to weight loss[6] and malnutrition in both hospitalized elderly[7] and psychogeriatric nursing home residents[8].

As incremental changes in metabolic rate[9] and anorexia present themselves early in disease states interventions may need to be started as early as possible in order to limit weight loss.

Therefore in the present study we investigated whether early use of a liquid nutrition supplement immediately after onset of acute illness from infection can prevent weight loss in elderly psychogeriatric nursing home residents.

Methods

Subjects. Subjects eligible for the study were psychogeriatric nursing home residents over 65 years of age who had stayed at the nursing home for at least 2 months since admission. Excluded were residents with cancer, rheumatoid arthritis, insulin dependent diabetes, morbid obesity, need for terminal care or for a therapeutic diet incompatible with supplementation. The study was approved by the Medical Ethical Committee of University Hospital Rotterdam. For all participants informed consent was obtained.

Design. The study had a randomized, placebo controlled prospective design. Residents were randomized to receive either early supplementation or standard treatment for 5 weeks after the onset of illness. Residents in the early supplementation group received 200 ml of a liquid nutrition supplement containing energy, vitamins and minerals (table I) daily besides their normal diet immediately after prescription of antibiotics by the physician. Intake of the supplement was noted by the nursing staff.

Table I*. Composition of supplement per 100 ml*

Energy		154.5	kcal
Carbohydrates		18.9	g
Fat		6.3	g
	DHA	4.6	mg
	EPA	111.7	mg
Protein		5.6	g
	total L-arginine	0.41	g
	total glutamine	1.04	g
Dietary fibre		2.25	g
Na		131	mg
K		197	mg
Cl		85	mg
Ca		57	mg
P		57	mg
Mg		24	mg
Fe		1.4	mg
Zn		1.5	mg
Cu		225	µg
Mn		450	µg
Se		13	µg
Mo		7.5	µg
Cr		5.0	µg
I		15	µg
F		150	µg
vitamin A		106	µg
β-carotene		1.5	mg
vitamin D		0.75	µg
vitamin E		21.7	mg α-TE
vitamin K		6	µg
vitamin C		55	mg
thiamin		0.15	mg
riboflavin		0.17	mg
niacin equivalent	2.97	mg	
pyridoxine		0.2	mg
vitamin B_{12}		0.3	µg
folic acid		20	µg
pantothenic acid		0.6	mg
biotin		15	µg
choline		30	mg
taurine		3.75	mg
carnithine		3.4	mg
myo-inositol		15	mg
nucleotides		57	mg

Dietary intervention in the standard treatment group started only once weight loss, loss of appetite and/or low intake was observed by the physician and the resident was referred to a dietitian. The dietitian prescribed food enrichment in line with wishes of the resident, which often included the supply of energy and protein enriched desserts or drinks.

Methods. Infection type and prescription of antibiotics were noted by the nursing home physicians. Weight in regular clothes without shoes was measured to the nearest 0.1 kg using a calibrated weighing scale. Height or knee height (if residents could not stand up) was measured by tape measure or kneemometer, respectively, to the nearest 0.1 cm and height was calculated from knee height[10]. Body mass index (BMI) was calculated (weight/height^2). Triceps skinfold thickness (TST) was measured with a skinfold caliper (Holtain, Dyfed, UK) at the left side of the body in triple to the nearest 0.2 cm[11]. Mid upper arm circumference (MUAC) and calf circumference were measured once at the left side of the body with a tape measure to the nearest 0.1 cm. Mid upper arm muscle circumference was calculated as MUAC – (0.314*TST)[12]. Dependency was measured by a questionnaire (Zorg Index Geriatrie (ZIG)) which measures need for care and care activities[13]. Increased dependency results in an increased ZIG score. The personal companion of the subject was asked to fill out the ZIG score list together with a nurse.
These parameters were measured by a trained investigator at the start of illness and 5 weeks afterwards.

Assessment of dietary intake. In the first and fifth week of disease, food intake (including the supplement) was assessed for three weekdays within one week by a combination of weighed and unweighed dietary records by a trained dietitian. The main meal was recorded by weighing the food if residents were served, and by estimating portion sizes from standard measures if residents served themselves. Leftovers of meals were weighed or the proportion of the leftover in relation to standard measures was estimated. The intake from other meals during the day and the supplement was recorded in terms of current household measures and standard portion sizes reported by nursing staff. Data were analyzed using a software program based on the Dutch food composition table of 1996.
Appetite and thirst were measured on a three-point scale as ‘decreased’, ‘similar’, or ‘increased’.

Statistics. Values are presented as means ± SD unless stated otherwise. Distribution of data was tested for normality by a Kolmogorov Smirnov test. Normally distributed parameters were compared using t-test. Not normally distributed variables (weight difference week 4 – week 0, MUAC difference week 4 – week 0, supplement group weight difference week 4 –

week 0) were tested with Wilcoxon's signed rank test. Distribution of dropouts was tested with Chi-square test. Intention-to-treat analyses of 38 residents including last-observation-carried-forward analysis for weight change did not result in different p-values or effect size. Data reported are thus for residents who have complete weight data for the disease period.
One-way ANOVA analysis was performed to correct weight change for age and baseline weight. A one-sided value of $p<0.05$ was considered significant. Data were analyzed with SPSS 10.0 for Windows (SPSS Inc.).

Results

Thirty-nine residents were randomized, of which 5 dropped out, 4 subjects because measurements after 4 weeks were not performed (3 standard, 1 supplement) and 1 subject because measurement of weight was not performed at the start of illness (standard). Distribution of dropouts between groups did not differ (p=0.604).

Table II reports resident characteristics at the start of disease for the total group and for the treatment groups. There were no significant differences in baseline parameters between treatment groups.

***Table II**. Baseline characteristics of psychogeriatric nursing home residents at onset of infectious disease (mean ± SD).*

	Total group	Standard	Supplement
N	34	16	18
Age (y)	82.7 ± 7.2	81.6 ± 7.5	83.8 ± 6.9
% female	85	81	89
BMI (kg/m^2)	24.5 ± 4.2	24.8 ± 4.9	24.4 ± 3.6
Location infection			
Urinary tract	19	10	9
Upper respiratory tract	3	2	1
Lower respiratory tract	9	4	5
Other	3	2	1

BMI = body mass index (kg/m^2)

Outcome parameters at baseline and changes during the disease period are reported in table III. The standard group lost 0.4 kg during illness, but this difference was not significant (p=0.477). The supplement group gained 0.8 kg during illness (p=0.081). Weight change during illness was significantly different between standard and supplement groups (p=0.040).

In analysis of variance age had no significant effect but weight before onset of disease did, which resulted in a p-value for changes of 0.033.

No differences were observed in changes of triceps skinfold thickness, calf circumference, mid upper arm circumference, energy and macronutrient intake between groups. After pooling the groups, no changes were observed in energy or macronutrient intake during the disease period. No changes in appetite and thirst were observed during the disease period.
In both groups the ZIG score increased by 1.5 point during the disease period (pooled p=0.008). There was no difference in change in ZIG scores between groups.

Discussion

We found an increase in weight after early nutritional supplementation immediately after diagnosis of acute illness from infection in elderly psychogeriatric nursing home residents. We detected no changes in nutritional intake over the disease period in either group. The required level of care increased during disease, but changes did not differ between groups.
Our finding of weight increase after nutritional supplementation was not sustained by other anthropometric measurements, as we observed no changes. However, the power of our study was probably not sufficient to detect any such changes. Bos *et al*.[14] studied weight change after short-term supplementation and reported increases in both energy intake and fat free mass. Their study was performed in 17 malnourished hospital patients with a lower BMI than observed in our study.
We studied residents with acute illness, defined as infection, as this is the most common acute illness in the elderly[5] and it only affects health in the short term, as opposed to e.g. stroke or fractures. In order to define infection, we decided to take antibiotic prescription as an objective criterion, as otherwise resident or staff reports of illness for e.g. a cold would be more subjective. This selection caused us to take an illness that has a relatively minor impact on long-term health, and potentially residents with other acute disease such as stroke might

also benefit from supplementation[15]. Besides, whilst we chose the most serious infections, an intercurrent weight loss from non-diagnosed infections may also be a problem in nursing home residents. These considerations indicate that the increase in weight in our study due to supplementation is relevant for a broader group of psychogeriatric nursing home residents and that effects observed on body weight might be even larger in malnourished residents.

We did not include a placebo group in our study, as we decided to test our intervention against current practice. This has probably not affected the direction of the effect of supplementation, as a placebo group would have received a non-caloric supplement instead of fortified meals, which would have increased the contrast. Besides the type of supplementation also timing of intervention differed between the supplement and standard group. Although not separated as such in our study, our results indicate that consistent early nutritional supplementation can achieve better results than intervention once (risk for) weight loss has already become evident. The difference in weight changes between supplement and placebo amounted to about 1 kg, which is a small, but relevant effect size in view of the short supplementation period.

We did not find a significant weight loss during illness from infection in our standard group. However, this group also received fortified meals, if such provision had not taken place, weight loss might have occurred. With regard to the causes of weight loss during disease, our study has only been able to investigate changes in nutritional intake during a disease period. A change was not detectable in this small group, as we found no effects of illness on appetite, thirst and energy intake.

The energy intake in our group of subjects is largely similar to that in other studies among residents in a nursing home[16,17]. Energy intake was low but did not change during the disease period. The contribution of the supplement was 300 kcal per day, which was relatively large compared to the daily intake (± 18%). Therefore an increase in weight may well have been expected and apparently this increased energy intake from the supplement was not completely compensated for through reduced intake of other food[18]. However in these small groups, we could not see this reflected in calculated energy intake.

Very few studies have investigated the short-term effect of nutritional supplementation in the elderly upon acute illness with infection. Woo *et al*.[19] found a similar weight change after provision of a liquid nutrition supplement for one month to elderly hospital patients with chest

infection. Also other studies during acute illness such a stroke[20] or admission to a hospital[14,21] reported positive effects of supplementation on body weight.

We conclude that early provision of a liquid nutrition supplement immediately after onset of acute illness from infection increases weight in elderly psychogeriatric nursing home residents.

Acknowledgments

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4

The effect of a liquid nutrition supplement on body composition and physical functioning in frail elderly.

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Submitted

Abstract

Background & aims: Elderly are at increased risk of poor nutritional status which is mutually interacting with functional status. We evaluated the effects of a liquid nutrition supplement on anthropometric and functional indices in frail elderly.

Methods: Subjects (n = 68; mean age = 82 ± 7 years) with body mass index ≤ 25 kg/m^2 received either a supplement or a placebo for 6 months. Anthropometric (body weight, bioelectrical impedance, calf circumference), biochemical (albumin, prealbumin), functional parameters (handgrip strength, timed 'up and go' test) and dietary intake were measured. Activities of Daily Living and Nottingham Health Profile were assessed.

Results: No compensation of energy intake occurred. After 6 months the supplement group had gained more weight (+1.6 kg) than the placebo group (+0.3 kg) (p=0.03). No other significant changes in anthropometric, functional or blood parameters were seen. There was a significant improvement on the section 'sleep' of the NHP (mean change ± SE = -0.38 ± 0.19 for supplement vs. 0.24 ± 0.19 for placebo, p=0.03).

Conclusion: Dietary supplementation led to an increase in body weight and had a positive influence on sleep in frail elderly persons. Supplementation did not affect energy intake from regular meals and thus resulted in additional energy intake.

Introduction

With advancing age, not only a loss of lean body mass is often observed but also a reduction in skeletal muscle mass, total body water and bone density and an increase in body fat[1]. It has been shown that the elderly are at increased risk of poor nutritional status compared to the general population[2,3]. Besides the effect of aging per se, malnutrition in the elderly is paired with a reduction in muscle strength and functional decline such as reduced walking time[4].

Our previous study in psychogeriatric nursing home patients showed that a liquid nutrition supplement increased body weight and improved nutritional status[5]. After these positive findings we extended our supplementation studies to free living elderly people, hereby including functional status and quality of life as primary outcome measures. In the absence of studies which failed to show an effect on functional status[6-8], we still hypothesized at the start of the present study that supplementation ultimately would either improve physical functioning as well as general well being or prevent a decline in these outcome measures.

It is thus important to know if nutritional supplementation in frail elderly can reduce the decline in functional status observed during aging. Thus the present study was designed to assess the effects of the use of a low volume liquid nutrition supplement on anthropometry, functional status and quality of life in a group of frail elderly people.

Subjects and methods

Study population. The study was a randomized, double blind 6-month intervention trial that included 101 elderly. Enrolment of subjects took place from May 1999 to August 2001. We selected frail Caucasian elderly people aged ≥ 65 y, based on a body mass index (BMI) ≤ 25 kg/m^2 and residency in a home for the elderly or sheltered housing. Subjects were not eligible when they had diagnosed cancer or chronic gastrointestinal disorders (Crohn's disease, colitis ulcerosa, stoma), when they needed a diet incompatible with supplementation or when they were mentally unable to answer the study questions or to remember taking the supplement. After obtaining written informed consent from each subject, participants were matched for BMI and randomized into supplement or placebo group. Treatments were allocated by a letter A to D by a person not involved in the study. The study protocol was approved by the Medical Ethical Committee of Wageningen University.

Intervention. The dietary supplement and the placebo product were provided in two flavors in 125-mL tetrapacks. The drinks had to be taken for six months two times a day in addition to

the normal food consumed, both in the morning and in the afternoon in between regular meals. The supplement consisted of a liquid nutrition drink, containing energy, protein, carbohydrate, fat, and micronutrients in amounts of approximately 30 to 150% of US RDA of vitamins and minerals, with increased levels of antioxidants. The supplement included per 100 mL 100 kcal (0.42 MJ), 3.5 g protein, 4.5 g fat, 11.4 g carbohydrates, 2.3 g fiber, 31 mg Na, 219 mg K, 15 mg Cl, 160 mg Ca, 160 mg P, 38 mg Mg, 3.8 mg Fe, 7.2 mg Zn, 0.8 mg Cu, 1.2 mg Mn, 20 µg F, 15 µg Mo, 34 µg Se, 11 µg Cr, 35 µg I, 80 µg RE vitamin A, 1.2 mg carotenoids, 90 mg vitamin C, 4 µg vitamin D, 22 mg-α-TE vitamin E, 20 µg vitamin K, 0.8 mg vitamin B1, 0.8 mg vitamin B2, 3 mg NE niacin, 1 mg pantothenic acid, 1 mg vitamin B6, 160 µg folic acid, 1.5 µg vitamin B12, 27 µg biotin, 1.2 mg coenzyme Q10 and 7.6 mg flavonoids. The placebo product contained water, sweetener, cloudifier, thickener, flavoring and colorant to resemble the dietary supplement in taste and appearance. Subjects received the drinks at home during visits every two weeks. Compliance was verified every two weeks as part of home interviews and by counting leftover supplements. Compliance percentage was quantified as (number of supplements provided – number of supplements returned / (number of days participation in study * 2)) * 100%.

Measurements

Data were collected at baseline and after 6 months of intervention.

Anthropometry. All anthropometric measurements were performed with subjects wearing light clothing without shoes and after visiting the toilet. Body weight was measured to the nearest 0.1 kg using a calibrated weighing scale (Seca, Germany). Knee height was measured in duplicate to the nearest 0.001 m using a stadiometer. Because a large proportion of the elderly were either unable to stand fully upright or had some degree of spinal curvature an estimation of height was calculated according to the following formula: height (cm) = 3.16 x knee height[9]. Body mass index (BMI) was calculated as weight in kilograms divided by squared estimated height in meters. Using a flexible measuring tape calf circumference was measured in duplicate to the nearest 0.001 m on the left side of the body, with the subject sitting with knee and ankle in a position of 90° Body composition was measured by bioelectrical impedance analysis (BIA) at the left side of the body with a Xitron 4000 bioimpedance analyzer (Xitron Technologies, San Diego, CA). Impedance was

measured at a frequency of 1 kHz (Z1) and 100 kHz (Z100). BIA was not measured in subjects when they had a pacemaker, an implant of steel or refused co-operation. Total body water (TBW) was calculated by using the equation[10]: TBW (kg) = 0.51301 x height^2/Z100* + 6.29. Fat free mass (FFM) was calculated as TBW/73*100; fat mass was calculated as body weight – FFM[10].

Physical functioning. Handgrip strength measurements were performed using a grip strength dynamometer (Jamar™ Model PC5030J1, Sammons Preston Inc., IL). Three consecutive measures of handgrip strength were recorded for both hands and the maximum strength effort was used in data-analysis.
Lower extremity functional strength was measured using the timed 'up & go' test. Three consecutive efforts of the timed 'up & go' test, according to standardized procedures[11], were recorded to the nearest 0.01 s with a stopwatch. Hereby the subject wore usual footwear and usual assistive device (cane or walker) was allowed during the test. The best effort was used for data-analysis.

Questionnaires. The Nottingham Health Profile (NHP) questionnaire consists of a list of questions concerning the subject's emotional reactions, pain, energy, sleep, social isolation, and physical mobility was used to assess this questionnaire. The total NHP score is obtained by the sum of all sections. A lower score indicates a better quality of life (range: 0-38)[12].
Ability to perform activities of daily living (ADL) was measured as an indicator of functional capacity. ADL was assessed according to a list of 16 items of daily activities (e.g. housework, use stairs)[13]. ADL was calculated as a sum score over all items. A lower sum score indicates better functional capacity (range: 0-64).

Dietary intake. A three-day (two weekdays and one weekend day) estimated dietary record was collected according to the method described by de Jong *et al*.[14]. Subjects filled out a diary which was checked by a dietician. Their nutrient intake from foods was calculated according to standard portion sizes using the computerized Dutch Food Composition Table of 1997[15].

Product acceptance. At the end of the study period, for each flavor the taste of the product was registered on a scale from 0 to 10. Average time to drink the supplement was registered.

Biochemistry. Blood samples were collected from fasting subjects between 0700 and 1000 h for all indicators. The samples were collected at home for practical reasons and immediately put on ice before further processing. Blood was collected in coagulation tubes of 10 mL and then separated by centrifugation at 3500 rpm and 4°C for 10 min. One mL serum was used to analyze the serum proteins albumin and prealbumin by Synchron Clinical Systems CX4CE using kits 442765 and 445855 (Beckman Instruments, USA). All these biochemical analyses were performed by the laboratory ABL, Assen, The Netherlands.

Statistics. Base-line characteristics of the two groups were compared using a Student's unpaired t-test or a Mann Whitney test. Statistical tests were considered to be significant at the two-sided $P<0.05$ level. Absolute changes ± SE were calculated for the intervention group and compared with the changes in the placebo group with a Student's unpaired t-test and for not normally distributed variables with a Mann Whitney test. As we hypothesized favorable effects of the supplement, statistical tests were considered to be significant at the one-sided $P< 0.05$ level. In each analysis the highest or best score for each functional indicator obtained from either side of the body and either session was applied. All statistical analyses were conducted in SPSS for Windows version 10.0 (SPSS Inc., Chicago, IL).

Results

At the beginning of the intervention 101 subjects were willing to participate. One subject of the placebo group died during the intervention. Eighteen subjects did not like or could not tolerate the supplement, 11 in the intervention group and seven in the placebo group. Ten subjects indicated that the study was too time-consuming, five in the intervention group and five in the placebo group. Four subjects withdrew because they were diagnosed with cancer, two in the intervention group and two in the placebo group. Finally, baseline and post-intervention measurements were available for 68 subjects. The participant flow is reported in Figure 1.

In Table 1 baseline characteristics of both study groups are reported. None of the baseline variables differed significantly between the two intervention groups, except for the timed 'up

and go' test. The subjects who received the supplement were slower than the subjects who received the placebo (p=0.041).

Table 2 reports changes in both groups over the study period. There was a significant difference in weight gain between the supplement group and the placebo group (p=0.031). The mean weight of the supplement group increased with 2.5% in contrast to a lesser weight gain of the placebo group (0.5%). Consequently there was also a significant difference in change in BMI between the two groups (p=0.011). These weight gains did not result in detectable changes in intracellular water, FFM, FM or calf circumference.

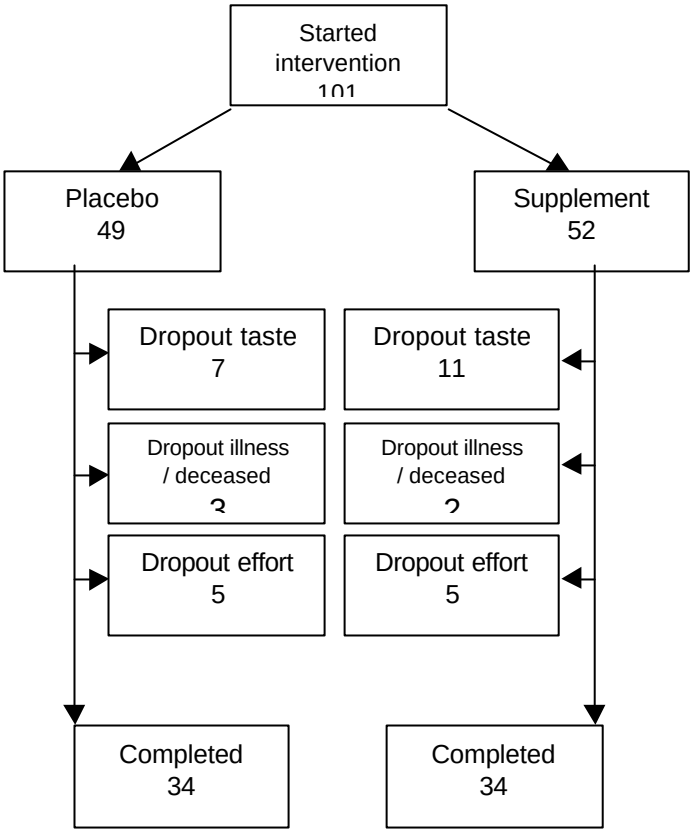

***Figure 1**. Flow chart of participation in the nutritional supplementation study*

***Table 1**. Baseline characteristics of frail elderly participants of the nutritional supplementation study (means ± SD).*

	Placebo group (n=34)	Supplement group (n=34)
Male, %	47	38
Age, y	81.0 ± 6.9	83.9 ± 6.3
Anthropometry		
Height, m	1.65 ± 0.009	1.64 ± 0.009
Weight, kg	66.7 ± 9.1	62.7 ± 8.3
Body mass index, kg/m^2	24.5 ± 2.3	23.4 ± 2.4
Calf circumference, cm	34.7 ± 2.6	33.4 ± 2.9
Total body resistance at 1 kHz,[1] Ohm	609 ± 92	633 ± 102
Total body resistance at 100 kHz,[1] Ohm	513 ± 79	531 ± 93
Fat free mass,[1] kg	47.9 ± 8.6	45.3 ± 9.4
Fat mass,[1] kg	31.0 ± 8.5	27.6 ± 10.5
Functional status		
Maximum handgrip strength, kg	25 ± 13	22 ± 9
Timed 'up and go', s	15.2 ± 5.7	22.6 ± 16.0*
Nottingham Health Profile	7.3 ± 5.3	9.9 ± 6.9
Activities of Daily Living	29.9 ± 8.1	31.5 ± 9.0
Nutritional intake[2]		
Energy intake, kcal/d	1586 ± 288	1724 ± 389
(kJ/d)	(6658 ± 1210)	(7232 ± 1624)
Protein, g/d	63 ± 12	67 ± 22
Fat, g/d	62 ± 15	74 ± 25
Carbohydrate, g/d	187 ± 43	191 ± 39
Water, g/d	1864 ± 478	1919 ± 475
Fiber, g/d	17.1 ± 4.0	17.0 ± 5.0
Biochemistry[3]		
Albumin, g/L	38.1 ± 2.9	38.2 ± 3.1
Prealbumin, g/L	0.22 ± 0.005	0.23 ± 0.006

[1] Bioelectrical impedance analysis for 41 subjects (23 placebo group; 18 supplement group).
[2] Dietary intake (excluding intake from the supplement) for 48 subjects (23 placebo group; 25 supplement group).
[3] Serum proteins for 47 subjects (24 placebo group; 23 supplement group).
* Differences are considered significant if two sided P < 0.05 vs. placebo group

No significant changes were observed for timed 'up and go', grip strength, ADL and total score on NHP. The 'sleep' section of the NHP questionnaire showed a significant difference between the supplement group and the placebo group (p=0.028). At the end of the intervention subjects in the supplement group slept better than before (mean change ± SE = -0.38 ± 0.19) in contrast to the subjects of the placebo group who had more difficulty with sleeping (mean change ± SE = 0.24 ± 0.19).

***Table 2**. Six months changes (means ± SE) in anthropometric and functional parameters in frail elderly after nutritional supplementation.*

	Placebo group (n=34)	Supplement group (n=34)	P-values[1]
Anthropometry[2]			
Weight, kg	0.3 ± 0.5	1.6 ± 0.4	0.031
Body mass index, kg/m^2	0.01 ± 0.2	0.57 ± 0.2	0.011
Calf circumference, cm	-0.1 ± 0.2	-0.2 ± 0.2	0.608
Total body resistance at 1 kHz, Ohm	14.7 ± 9.9	7.1 ± 19.5	0.643
Total body resistance at 100 kHz, Ohm	2.9 ± 5.7	-6.2 ± 13.6	0.255
Functional status			
Maximum handgrip strength, kg	-0.4 ± 1.0	-0.7 ± 1.0	0.605
Timed 'up and go', s	0.2 ± 0.8	1.1 ± 1.0	0.619
Nottingham Health Profile	0.1 ± 0.6	-0.8 ± 0.5	0.102
Activities of Daily Living	0.2 ± 0.7	1.0 ± 0.8	0.854
Nutritional intake[3]			
Energy intake, kcal/d (kJ/d)	-24 ± 54 (-101 ± 227)	-121 ± 87 (-503 ± 364)	0.179
Protein, g/d	-3 ± 3	-3 ± 3	0.321
Fat, g/d	1 ± 3	-8 ± 5	0.068
Carbohydrate, g/d	-6 ± 7	-8 ± 10	0.423
Water, g/d	-19 ± 74	-139 ± 120	0.202
Fiber, g/d	1.4 ± 0.7	-0.6 ± 1.3	0.073
Biochemistry[4]			
Albumin, g/L	-0.8 ± 0.7	-0.3 ± 0.7	0.310
Prealbumin, mg/L	-0.004 ± 0.001	0.003 ± 0.001	0.350

[1] Differences are considered significant if one-sided $P < 0.05$.
[2] Bioelectrical impedance analysis for 41 subjects (23 placebo group; 18 supplement group).
[3] Dietary intake (excluding intake from the supplement) for 48 subjects (23 placebo group; 25 supplement group).
[4] Serum proteins for 47 subjects (24 placebo group; 23 supplement group).

Energy intake from regular foods did not change significantly over the study period within either of the two groups nor was there a difference between groups in changes over the study period. Therefore when including the contribution of the supplement, total energy intake increased in the supplement group over the study period.

We observed no significant changes in albumin and prealbumin due to supplementation.

Intention to treat analysis (last observation carried forward) of the 101 subjects who started the study showed no differences with the analysis of the group described above. The same variables were significantly different between the two groups i.e. weight (p=0.020) and BMI

(p=0.005). Also the variable NHP-sleep showed the same significant difference between the two intervention groups (p=0.029).

There was no significant difference in compliance between the subjects who received the supplement or the placebo. A high compliance was seen for the supplement group (85 ± 36%) as well as for the placebo group (94 ± 24%). The two flavors of the supplements were equally appreciated (p=0.477). The total appreciation for the supplement in the supplement group was 7.5 ± 1.6 and for the placebo 7.0 ± 1.0. The majority of subjects (94%) did not have problems to directly consume the 125-mL package and the median time this took was one minute. Only 20% of subjects reported that it took some effort to drink two packages per day.

Discussion

In the present study nutritional supplementation in elderly subjects had a positive effect on weight and the section 'sleep' of the Nottingham Health Profile, but no other effects on the selected anthropometric or functional measurements were detected.

There was no significant difference found in compliance between the subjects who received the supplement or the placebo. In the beginning of the study some subjects dropped out but the baseline characteristics between the dropouts and the elderly who finished the study did not significantly differ. Therefore no selective withdrawal occurred.

We wanted to study a free living elderly population at risk of malnutrition and therefore we approached subjects living in sheltered housing or homes for the elderly, and one of the criteria for entering the study was a BMI value $\leq$ 25 kg/m^2. The anthropometric values and energy intake of our frail subjects are comparable to those measured in the study of frail elderly by de Jong *et al*.[14]. Our study consisted of frail elderly persons who could perform most daily activities by themselves but did need a certain amount of care. In comparison with other studies the subjects of the present study seem to have a health profile that is worse than that of apparently healthy Dutch elderly people. The Dutch elderly population of the European Seneca study[16] had a higher mean BMI value, higher physical activity level and a higher daily energy intake.

Energy intake in our study was low, 29% of subjects had an intake below 1500 kcal which means they are at increased risk of deficiencies[17]. When providing a supplement there is a risk that its intake will replace nutrient intake from normal foods. We therefore asked subjects to consume the liquid nutrition supplement between main meals. We did not observe a

significant decline in energy intake from regular food in the supplement group, while our study had sufficient power to show a meaningful difference in energy intake in the range of the amount present in the supplement (250 kcal). To establish a significant difference of as low as 100 kcal per day between two intervention groups we would have needed 320 subjects. Our observation of a lack of compensation is comparable to other studies[18-20]. Thus provision of a liquid nutrition supplement leads to an increase in total energy intake, which is sustained by the increase in weight observed in our study.

Our findings are in agreement with those of Gray Donald *et al*.[6] who found a weight gain after a 12-wk intervention in supplemented frail elderly subjects. Like in our study no functional changes were observed. It was suggested that the intervention period was too short. However the 6-month intervention in our study also showed no effect on functional variables, and thus potentially an even longer supplementation time would have been required. A recently reported 18-month study on nutritional supplementation and/or exercise in elderly assigned to public outpatient clinics showed maintenance in functional status in supplemented patients[7]. Activities of daily living scores remained constant in subjects who received the supplement but declined in those who did not, whereas for other functional indices no effect of nutritional supplementation was observed. A significant increase in FM after 18 months was recorded but in contrast to most studies (including our study) body weight remained constant during the observation period. The failure of finding improvements in body weight can be ascribed to the low compliance seen in their study. The study population received the supplement as a soup or porridge and given as two daily snacks. It could be that the supplement was taken as a replacement of the normal daily intake, therefore no extra energy was available to promote a weight gain, this can not be checked as nutritional intake was not measured.

Bioelectrical impedance analysis was chosen to measure body composition. A disadvantage of the method is that relatively large numbers of subjects are required because individual errors are large[21,22]. We found no significant changes in FFM due to supplementation. If theoretically all of the weight gained (1.3 kg) would have been fat free mass, we would have needed 162 subjects to demonstrate a significant change, thus the power in our sample size (n=68) was too small to detect a change in body composition. Like in our study Fiatarone *et al*.[23] did not find a significant effect of supplementation on total body water as measured by bioelectrical impedance. We expected that if an increase in FFM occurred, this could result in improvements in functional status of the elderly subjects. However, no functional

improvements were recorded during the intervention. An explanation for this lack of effect could be that the increase in weight should be attributed to FM although our study method did not allow to verify this. We measured calf circumference as a measure of muscle mass as this is preferred over mid upper arm muscle circumference in the elderly[24]. Also for this parameter we did not observe changes, and no supplementation studies have previously used calf circumference as outcome parameter of an intervention.

As in the present study no functional improvements were noted, it is possible that the parameters chosen to measure functional status were not sensitive enough to change. The methods used are able to differentiate between elderly who are in good health and those who are not[25-29]. However, a ceiling effect may be present, as once elderly loose the habit of performing an activity, they may not restart the activity unless a large improvement in their health status has occurred. Such an effect may explain the fact that improvement on ADL[30] and handgrip strength have been observed after supplementation of undernourished geriatric patients during hospitalization[31].

It could also be that exercise is needed to induce functional effects. If the elderly are no longer used to performing (heavy) physical activities they may not be trained to use their increased force potential. After a 17-wk exercise intervention in an elderly population an increase in FFM was found but no additive effect of a micronutrient supplement was seen[14]. An intervention consisting of an energy-dense supplement and an exercise program could perhaps induce an additive effect of the supplement given. In three interventions where an energy-dense supplement was combined with an exercise program only an increase in FM was seen and no functional improvements were noted[7,23,32]. Bunout *et al*.[7] did not measure daily energy intake and Fiatarone *et al*.[23] found a decrease in total energy intake. So an explanation might be that no extra energy was available to provoke an increase in energy expenditure. This is sustained by the reporting of Meredith *et al*.[32] that changes in anthropometric values were proportional to changes in energy intake. Therefore a study on the combined effect of increased energy intake and exercise would still be required to confirm this hypothesis.

After the 6-month intervention period subjects who received the supplement slept better. The effect on sleep could be due to the use of multiple comparisons, but is consistent with a study done in elderly with persistently low thiamin pyrophosphate concentrations when providing thiamin supplements[33]. A study in free living Irish women showed significant improvements in

appetite, fatigue, and general well being and a trend towards an improvement in sleep after thiamin supplementation[34]. The supplemented group needed less daytime sleep and they had less difficulty in falling and staying asleep at night. In contrast to the study of Wilkinson *et al*.[33] their study did find an increase in weight after supplementation. This weight gain was the effect of an increase in appetite. In our study we did not measure appetite, but as intake from regular foods did not change over the study period it may have increased.

Baseline measurements indicate that the blood values of albumin and prealbumin found in our subjects were normal. We did not find a change in albumin and prealbumin during the intervention. The literature about a change in biochemical indices after supplementation is contradictory. In several studies the biochemical indices did not change after nutritional supplementation[8,31,35-37]. When changes did occur the population chosen consisted of elderly with a very bad health profile which is accompanied by nutritional deficiencies and low blood values of albumin and prealbumin[38-41].

In conclusion, we found that dietary supplementation led to an increase in body weight and had a positive influence on sleep in frail elderly persons, but no functional improvements could be observed. Supplementation did not affect energy intake from regular meals and thus contributed to total energy intake.

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5

Effect of an enriched drink on cognitive function in frail elderly people.

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Abstract

Background: Many elderly people indicate having difficulties to learn something new and to remember names, plans and conversations. As the decreased cognitive function in elderly people is potentially related to their poor nutritional status, provision of essential nutrients may improve cognitive function.

Objective: To investigate whether consumption of an enriched drink including moderate doses of all essential micronutrients can improve cognitive function in frail elderly people.

Design: Randomized, double blind, placebo-controlled trial. Frail Caucasian elderly people (*n*=101) aged ≥65 years and with a body mass index (BMI) ≤25 kg/m^2 were selected. Subjects received either an enriched drink or a placebo product for 6 months. Before and after the intervention, assessments of cognitive function (word learning test (WLT), WLT delayed, category fluency (CF) for animals and professions and recognition memory test for words (RMTW)) and blood biochemistry (vitamin B12, homocysteine) were conducted.

Results: 67 Residents completed the study period. After 6 months, there was a significant difference in changes of the WLT (0.9±0.3 vs. -0.1±0.3; p=0.014) and CF professions (1.2±0.7 vs. -0.6±0.5; p=0.017) in the supplement group (*n*=34) compared to the placebo group (*n*=33). No significant differences were observed in WLT delayed, RMTW and CF animals. There was a concurrent significantly different increase in plasma vitamin B12 concentration (105±50 vs. 8±16; p=0.003) and decrease in homocysteine concentration (-6.3±5.9 vs. -0.3±2.9; p=0.000) in the supplement group compared to the placebo group.

Conclusions: Our study contributes to the evidence that nutritional supplementation may improve neuropsychological performance in frail elderly people.

Submitted

Introduction

Many elderly people indicate that their ability to learn something new (68%) and to remember names (54%), plans (48%) and conversations (35%) has decreased compared to the past[1]. It is estimated that 22% of the community-dwelling elderly is cognitively impaired. This proportion increases with age[2]. Several studies found that older adults' performances on a variety of psychometric tests decline over time (reviewed by Storandt[3]). Specifically declarative memory, a domain of memory associated with conscious recollection and usually assessed with recall or recognition tests, is consistently impaired in elderly people[4].

The causes of cognitive impairment in elderly people are multiple[2], and impaired nutritional status has been mentioned as one of them[5]. Through epidemiological studies the presence of an association between nutrition and cognitive function has become more clear over the past decades[6,7]. These studies have shown that low intakes of nutrients such as thiamin, vitamin B6, vitamin B12, folate[8-12], antioxidants[10,13-15] and specific fatty acids[16] are related to a decreased cognitive function.

Elderly people are at risk of deficiencies in the above mentioned nutrients. Low food intake is prevalent in certain groups of elderly people, with the highest prevalence in institutionalized elderly[17]. This results in a prevalence of inadequate intake of vitamin B1, vitamin B2, vitamin B6 and vitamin C of 61%, 31%, 58% and 18%, respectively[17], confirmed by suboptimal biochemical concentrations. Besides institutionalized elderly people, free living frail elderly people are at risk of such impaired nutritional Status.

As decreased cognitive function in elderly people is potentially related to their poor nutritional status, provision of essential nutrients may improve cognitive function. To date, few supplementation studies aimed to improve cognitive function have been performed. Some of these were studies without placebo treatment[18], used subjects who already had clinical symptoms of cognitive impairment[19-22] and/or had biochemical deficiencies[21,23,24]. Only few studies have been performed in healthy community-dwelling elderly people of which results were contradictory[25,26] or results were positive but only in these studies in which megadoses of micronutrients were supplied[27-29].

Therefore, the aim of this study was to investigate in a well-designed study whether use of an enriched drink including moderate doses of micronutrients can improve cognitive function in frail elderly people. To explore a potential mechanistic background of changes in cognitive function, plasma homocysteine and vitamin B12 concentrations were also measured.

Subjects and methods

Study design. In this randomized, double blind, placebo-controlled trial, subjects received either an enriched drink or a placebo product for 6 months. Before and after the intervention assessments of cognitive function and blood biochemistry took place.

Subjects were randomly assigned in blocks of 4, matched for BMI to receive either nutritional supplementation with a 125 ml enriched drink (containing 30-150% of US RDA of vitamins and minerals, with enhanced amounts of antioxidants, and containing 250 kcal energy in a daily dose)(Table 1) or a non-caloric placebo, twice daily between main meals. Subjects were randomly assigned to receive either a supplement or a placebo by an independent person not involved in the study. To maximize compliance, subjects were visited at their homes every 2 weeks. At this time they were supplied with additional supplements and any unused packages were counted. Compliance percentage was quantified as (number of supplements provided – number of supplements returned / (number of days participation in study * 2)) * 100%. All tests were administered by the same trained investigator before and after 6 months of supplementation.

Subject recruitment. We selected frail Caucasian elderly people aged ≥65 years, based on having a body mass index (BMI) of less than 25 kg/m^2 and on residency in a home for the elderly or sheltered housing residence. Residents with cancer, gastrointestinal disease, need for a therapeutic diet incompatible with supplementation or mental inability to respond to study questions or to remember taking the supplement were not eligible to participate. Subjects were enrolled between May 1999 and March 2001. The study was approved by the Medical Ethical Committee of Wageningen University, The Netherlands. Participants gave written informed consent before randomization.

Methods

Subjects characteristics. Age, sex, number of prescribed medicines, diagnosed chronic diseases and visual impairment were recorded. The level of education was assessed based on the number of years of education and grouped in 3: 6 years or less, 7 to 9 years or more than 9 years.

Dietary intake. A three day (two weekdays and one weekend day) estimated dietary record was collected according to the method described by de Jong *et al*.[30]. Subjects filled out a diary, which was checked by a dietician. Food intake was calculated using standard portion

***Table 1**. Composition of the dietary supplement per daily dose (250 ml)*

Nutrient	
Energy (MJ) (kcal)	1.05 (250)
Protein (whey)(g)	8.75 (14 energy%)
Carbohydrates (g)	28.5 (46 energy%)
Fat (g)	11.3 (40 energy%)
Dietary fibre (g)	5.8
Na (mg)	78
K (mg)	548
Cl (mg)	38
Ca (mg)	400
P (mg)	400
Mg (mg)	95
Fe (mg)	9.5
Zn (mg)	18
Cu (mg)	2
Mn (mg)	3
F (µg)	50
Mo (µg)	38
Se (µg)	85
Cr (µg)	28
I (µg)	88
Vitamin A (µg)	200
carotenoids (mg)	3
Vitamin D (µg)	10
Vitamin E (mg)	55
Vitamin K (µg)	50
Vitamin C (mg)	225
Vitamin B1 (mg)	2
Vitamin B2 (mg)	2
Vitamin B6 (mg)	2.5
Vitamin B12 (µg)	3.8
Niacin (mg NE)	7.5
Pantothenic acid (mg)	2.5
Folate (µg)	400
Biotin (µg)	68
Coenzyme Q10 (mg)	3
Flavonoids (mg)	19

Placebo: no energy, no vitamins, no minerals

sizes with the computerized Dutch Food Composition Table of 1997[31].

Blood sampling. Venous blood samples were collected in coagulation tubes (vitamin B12) or heparinized tubes (homocysteine) at baseline and at the end of the study, after an overnight fast in the subjects' homes while they were in a sitting position. Samples were cooled immediately after collection and were centrifuged within 4 hours at 2500x*g* for 10 min at 4°C. After centrifugation, plasma (homocysteine) or serum (vitamin B12) was removed and

stored at –80°C for batch analysis at the end of the study.

Biochemical measurements. Plasma homocysteine was measured by HPLC according to Daskalakis *et al*.[32]. Serum vitamin B12 was analyzed in a selected group of 26 subjects of whom leftover serum samples were available using ion capture Im_x (Abbott Labs, Abbott Park, IL, USA)[33]. Concentrations above 221 pmol/l were considered normal[34]. Subjects who received vitamin B12 injections were excluded from the serum analysis. Analyses were performed at the Department of Clinical Chemistry, University Medical Center St. Radboud, Nijmegen (vitamin B12) and Department of Analytical Chemistry, Numico Research, Wageningen, The Netherlands (homocysteine).

Anthropometry. Anthropometric measurements were performed with subjects wearing light clothing without shoes and after visiting the toilet. Body weight was measured to the nearest 0.1 kg using a calibrated weighing scale (Seca, Germany). Knee height as an estimate of height was measured to the nearest 0.1 cm using a stadiometer. BMI was calculated as weight/(knee height^2*10)[35].

Mini mental state examination (MMSE). The Mini Mental State Examination was performed according to the method of Folstein & Folstein[36]. This is a scored series of eleven questions concerned with orientation, memory, attention, and the ability to follow verbal and written commands. It has a high reliability, is significantly correlated with more sophisticated tests of neuropathology, and distinguishes between people with or without cognitive disturbances. A maximum score of 30 points for persons in good mental state can be achieved[37] and a cut-off score of 23 was used to define cognitive impairment[38]. The version including the serial sevens form was selected[39].

Geriatric Depression Scale (GDS). The short version of the geriatric depression scale for affective capacity[40] is a simple self-rating 15 item questionnaire requiring yes or no answers[41]. Subjects were classified as depressed if they had a score higher than 5.

Neuropsychological tests

Word learning test (WLT). The Dutch version of the 15-word learning test was administered (Department of Neuropsychology, University of Groningen, The Netherlands). Subjects were presented verbally 15 words subsequently, and were asked

to repeat as many words as possible (immediate recall). This was repeated 5 times, and a delayed recall (WLT delayed) was asked after performing non-strenuous non-neuropsychological tests for 10 minutes. Total number of correct words of the 5 repetitions was summed and divided by the number of repetitions (maximum score was thus 15). Delayed recall was scored as the total number of correct words (maximum 15).

Category fluency test (CF). Two category fluency tests were administered in which subjects were asked to produce, within 60 seconds, as many animals or professions as they could think of[42,43]. The total number of items named within each category (excluding repetitions) was scored.

Recognition memory test for words (RMTW). The Dutch version of the recognition memory test for words as described by Diesfeldt[44] was used. After presentation of 50 words, spaced by 3 seconds, subjects were asked to recognize the words from 50 pairs including a correct word and a distractor. The score was counted as number of correct recognitions (maximum 50).

Statistical analyses. Values mentioned are means ± SD for descriptives, when changes are compared means ± SEM are shown. The characteristics and baseline neuropsychological scores of the supplement and control groups were compared by two-sided Student *t* test, Chi square test and Wilcoxon's signed rank test (if not normally distributed according to Kolmogorov Smirnov test: WLT delayed, CF animals). The changes in neuropsychological scores in both trial groups were compared by one-sided Student *t* test as we hypothesized improved cognitive function in the supplement group. Post-hoc tests were performed by means of paired t-tests. P values < 0.05 were considered significant. Effect size (ES) was calculated as mean change/SD[45]. Data were analyzed with SPSS 10.0 for Windows (SPSS Inc.).

Results

Baseline characteristics and study compliance. One hundred and one residents were randomized, of which 34 dropped out during the study period. A participant flow chart is reported in Figure 1. Intention-to-treat-analyses of the 101 residents using last observation carried forward analysis for outcome variables did not differ from subjects who completed the study. Data reported are thus from residents for whom complete data for the study period

***Table 2**. Baseline parameters of frail elderly participating in the supplementation study (means ± SD).*

	Placebo	Supplement
N	33	34
Age (y)	81 ± 7	84 ± 6
% female	53	62
BMI (kg/m^2)	24.1 ± 2.3	23.5 ± 2.4
Energy intake (kJ/d)[1]	6818 ± 1210	7133 ± 1636
(kcal/d)	1623 ± 289	1700 ± 392
Intake vitamin B1 (mg/d)[1]	0.94 ± 0.77	1.04 ± 0.94
Intake vitamin B2 (mg/d)[1]	1.23 ± 0.37	1.19 ± 0.35
Intake vitamin B6 (mg/d)[1]	1.13 ± 0.28	1.11 ± 0.33
Intake vitamin B12 (µg/d)[1]	3.59 ± 1.50	3.71 ± 2.35
Intake folate (µg/d)[1]	203 ± 52.7	182 ± 61.2
GDS[2]	3.7 ± 2.5	3.3 ± 2.4
> 5 (%)	21	12
MMSE[3]	26 ± 3	26 ± 3
≤ 23 (%)	21	24
Education (%)		
≤ 6 years	38	50
7-9 years	47	35
> 9 years	15	15
Impaired vision	1/33	4/34
No. of chronic diseases	1.6 ± 1.1	2.0 ± 1.4
No. of medications	3.6 ± 2.8	4.2 ± 3.7

[1] *n*=25+27 for both groups

[2] Geriatric Depression Scale

[3] Mini Mental State Examination

have been obtained. Baseline characteristics were similar in the group that completed the study period and the group that dropped out, except for the MMSE, which was significantly lower in the group that dropped out. A high compliance was observed for the supplement group (88 ± 23 %) as well as for the placebo group (94 ± 14 %).

Table 2 reports characteristics at baseline for both groups. There were no significant differences in characteristics between treatment groups. Twenty-three percent of subjects had a score of 23 or less on MMSE, which indicates increased risk of dementia or mild cognitive impairment. Sixteen percent of subjects had a score higher than 5 on the GDS, indicating the presence of depression. The proportion of subjects with a vitamin intake below 2/3 of the US RDA[46] were 36 % for vitamin B1, 11 % for vitamin B2, 53 % for vitamin B6, 10 % for vitamin B12 and 15 % for folate.

Neuropsychological tests. The neuropsychological scores at baseline and follow-up are shown in Table 3.

There was a significant improvement on the WLT (ES=0.43) and CF professions (ES=0.44) over the study period in the supplement group (*n*=34) compared to the placebo group (*n*=33). No significant differences were observed for changes between groups in WLT delayed (ES=0.20), RMTW (ES=-0.39) and CF animals (ES=0). The supplement group had significantly lower baseline WLT and WLT delayed. Paired t-tests indicated a significant increase in scores in the supplement group for WLT, WLT delayed, CF professions and CF animals but not for RMTW. No significant changes occurred in the placebo group.

Plasma vitamin B12 and homocysteine. There was a significant increase in vitamin B12 concentration and a decrease in homocysteine concentration in the supplemented group over the study period (Table3). Concentrations remained similar in the placebo group. This resulted in a significant difference between groups in change over the study period.

Discussion

We observed a significant improvement on two tests of cognitive function (WLT and CF professions) upon 6 months consumption of an enriched drink by frail elderly people.

The population we selected were elderly people living in sheltered housing and elderly homes who would be able to respond to study questions and who would not forget daily intake of the supplement. Although they were not severely cognitively impaired, the presence of subjects with mild cognitive impairment or dementia cannot be excluded, as it can be present at an early stage without being diagnosed, which is suggested by the 23% of subjects with low MMSE scores. Potentially participation of subjects with very high or very low cognitive function was relatively low, which resulted in participation of subjects with an

***Table 3**. Mean scores on memory tests at baseline and changes after 6 months of nutritional supplementation in frail elderly receiving placebo (n=33) or supplement (n=34).*

	Baseline (mean ± SD)		Change (mean ± SEM)		*P*	95% CL
	Placebo	Supplement	Placebo	Supplement		
Neuropsychological tests						
WLT[1]	6.1 ± 2.2	4.4 ± 2.1*	-0.1 ± 0.3	0.9 ± 0.3	0.014	-1.71
WLT delayed[2]	6.6 ± 2.1	4.7 ± 2.8*	0.3 ± 0.4	0.9 ± 0.4	0.152	-1.85
RMTW[3]	40.9 ± 5.5	40.3 ± 4.9	0.7 ± 1.2	-1.1 ± 0.9	0.383	-1.19
Category fluency animals	15.2 ± 4.3	13.9 ± 4.5	0.9 ± 0.7	0.9 ± 0.6	0.473	-1.88
Category fluency professions	11.9 ± 3.4	10.1 ± 4.3	-0.6 ± 0.5	1.2 ± 0.7	0.017	-3.46
Biochemistry						
Plasma homocysteine (µmol/L)[4]	17.6 ± 5.0	18.4 ± 7.9	-0.3 ± 2.9	-6.3 ± 5.9	0.000	-
Plasma vitamin B12 (pmol/L)[5]	290 ± 99	304 ± 118	-8 ± 16	105 ± 50	0.003	-

* P<0.01 vs placebo

[1] Word learning test

[2] *n*=27 for both groups

[3] Recognition memory test for words; *n*=30 placebo, 28 supplement

[4] *n*=23 placebo, 22 supplement, nonparametric test

[5] *n*=14 placebo, 12 supplement, nonparametric test

intermediate cognitive function. We observed a selective dropout of subjects with a low MMSE score. The final mean MMSE score in our study population is comparable to scores found in the general elderly population (mean MMSE 26)[38], slightly higher than the average found in elderly home residents (mean MMSE 20)[18], elderly people living in service flats (mean MMSE 23)[47], and frail elderly people living at home (mean MMSE 20)[48] but lower than in healthy elderly people (mean MMSE 28-29)[49,50]. Chandra *et al*.[25] observed a lower MMSE score in subjects with a plasma deficiency of one or more vitamins compared to those who were not deficient. As a significant proportion of our subjects had low intakes of vitamins and the average homocysteine concentration (which can be considered as an indicator of impaired status of vitamin B6, vitamin B12 and folate[51]) in our population was higher than in other studies of free living elderly people[26,34,52]. This supports the assumption that our population is at risk of subclinical deficiency and therefore potentially benefiting from supplementation.

Since plasma homocysteine concentrations are related to cognitive function[53] we measured changes in homocysteine levels as a biochemical variable besides the cognitive tests. Indeed alongside the improvement in cognitive tests we found a significant decline in plasma homocysteine concentrations. Our data support the efficacy of relatively low dose oral micronutrient supplementation for lowering homocysteine concentrations in elderly people, as found in a previous studies in other groups of elderly people[26,54]. However, changes in antioxidant levels (unpublished results) may also have contributed to the improvement on the neuropsychological tests.

The tests we used in the present study were representative for the areas of cognitive decline in elderly people[1,55,56]. Verbal fluency reflects frontal lobe and language function[57] and studies have suggested that vitamin B12 deficiency may be associated with frontal lobe damage[58]. Antioxidant levels appear to be related to the same tests of semantic memory, free recall and recognition[15]. Previous supplementation studies showed that the tests we used were sensitive to improvements[24].

We used the same test-versions at both measurement times, because we did not expect the presence of a learning effect after such a long time period. Moreover, the study was placebo controlled. Paired analysis in the placebo group indeed showed no significant changes over the study period for any of the neuropsychological tests. This is in line with the observations by Chandra *et al*.[25] over a one year period.

The score on the WLT is a measure of short-term memory, and the delayed score a measure of intermediate memory[59]. The absolute scores on the WLT in our population were much

lower than in healthy elderly people with a mean age of 81 years[60]. The effect sizes we found on the WLT and CF professions can be considered between small to medium according to the classification of Cohen[45]. This means that the results are, from a statistical point of view, substantial and point to a possible clinical relevance.

The fact that the baseline scores on two of the neuropsychological tests were not well matched for our groups may affect the interpretation of our results. It could be that if scores in the supplement group had been similar to the placebo group, no effects of supplementation would have been observed. However, scores in the placebo group were not as high to preclude improvement, rather, the lower scores in the supplement group may have risked to preclude improvement. Subtle changes in cognitive function can be difficult to measure due to impossibility for improvement on neuropsychological tests in more severely impaired individuals. A possible explanation for this could be that restoration of the transmethylation capacity with increased synthesis of neurotransmitters could contribute to the beneficial effect of vitamin substitution. As observed in a study with demented patients, mildly to moderately demented patients can be considered to have better preserved neuronal function and are more likely to respond clinically to vitamin substitution than severely demented patients[21]. As also indicated by the MMSE scores, we did not include such a severely impaired study group and the scores in the supplement group did increase over the study period. Chandra *et al*.[25] observed that those subjects whose baseline plasma values were low and responded to supplementation had a larger improvement in cognition. This may have been the case for our supplement group, as low plasma concentrations of several vitamins were observed in this group.

Comparison of our study with other studies is influenced by differences in methodology. These differences are related to the study population, cognitive tests, type of micronutrient(s) and dosage used. Our positive findings are therefore the result of the selection of frail elderly people, provision of adequate doses of a range of micronutrients, sufficient duration, sensitive tests and a relatively large sample of subjects.

This study contributes to the evidence that nutritional supplementation may improve neuropsychological performance in frail elderly people. From the effect sizes we obtained we may infer that our results have clinical importance.

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6

Biochemical antioxidant levels respond to supplementation with an enriched drink in frail elderly people

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Abstract

Objective: To investigate whether a drink enriched with essential vitamins and minerals can improve biochemical status of enzymatic and non-enzymatic antioxidants in frail elderly people.

Methods: *Design.* A 6-month randomized, double blind, placebo controlled intervention study. *Subjects.* Frail elderly people aged ≥ 65 years, with a body mass index (BMI) below 25 kg/m^2 and residency in a home for the elderly or in sheltered housing. *Interventions.* Enriched (with essential vitamins and minerals in 30-150 % of RDA and higher levels of antioxidants) drink (n=28) or placebo (n=27) to be taken twice a day in addition to the normal food consumed. *Measures of outcome.* Plasma levels of vitamin C, vitamin E, antioxidant capacity (TEAC), cysteine, uric acid and whole blood levels of total thiol and glutathione peroxidase (GSH-Px), dietary intake.

Results: Changes in vitamin E (16 ± 2 vs. 2 ± 1 μmol/L), vitamin C (37 ± 5 vs. 1 ± 5 μmol/L), TEAC (38 ± 15 vs. -10 ± 11 μmol/L Trolox eq) and cysteine (17 ± 10 vs. 0.4 ± 6 μmol/L) were significantly different between groups (p<0.05). There was a trend towards significant changes in erythrocyte glutathione peroxidase (-0.2 ± 3 vs. -10 ± 7 U/mg Hb; p=0.097). Baseline dietary intake of antioxidant vitamins was below 2/3 RDA for a substantial proportion (14-48%) of subjects.

Conclusions: We conclude that supplementation with an enriched drink can raise plasma levels of enzymatic and non-enzymatic antioxidants in frail elderly people.

Submitted

Introduction

According to the free radical theory of aging, free radicals play an important role in the aging process, and contribute to many common diseases[1]. Data provided by cross-sectional, case-control and prospective epidemiological studies raise supportive arguments for the relation between the intake of antioxidant vitamins and trace elements, and the risk of pathologies[2-5]. Therefore, controlling free radicals may be important for health maintenance in elderly people.

The antioxidant defense system comprises a number of interconnected, overlapping components that include both enzymatic and non-enzymatic factors. Vitamin E, the major lipid-soluble antioxidant, protects against lipid peroxidation. Vitamin C can quench free radicals as well as singlet oxygen, and can also regenerate the reduced antioxidant form of vitamin E. Together with uric acid, carotenoids, flavonoids and ubiquinol, these antioxidants make up the total antioxidative capacity (TEAC) in plasma. Some metals (zinc, copper, manganese, selenium) exert their actions as antioxidants via their incorporation into specific enzymes e.g. superoxide dismutase (SOD), which catalyzes dismutation of the superoxide anion into hydrogen peroxide, and glutathione peroxidase (GSH-Px), which detoxifies hydrogen peroxide and converts lipid hydroperoxides into nontoxic alcohols. Glutathione assists in the synthesis of protein and DNA, the maintenance of intracellular thiol groups, the enzymatic reduction of dehydroascorbate, the transport of amino acids into cells and the elimination of toxic compounds[6]. Cysteine is a precursor for glutathione formation.

The dietary intake of the antioxidant micronutrients can modulate the activity of the defense system and thus impact on the degree of protection provided to the cell or tissue against oxidative reactions. The intake of the non-enzymatic antioxidants vitamin C and sometimes vitamin E is lower in elderly people than in younger adults[7], and plasma levels are low[7-9]. The most frequently investigated enzymatic antioxidant in elderly people is GSH-Px. Selenium is an important component of this enzyme and plasma selenium status is reduced with aging[10,11]. It seems that in elderly people the GSH-Px status is largely related to their health status[7,12-16], potentially through a relation with selenium status[11,17,18]. Also levels of glutathione have been found to be reduced in elderly people[13,19,20] and reduced levels have also been related to presence of illness[20,21].

Several studies investigated the effects of micronutrient supplementation on plasma antioxidants in different groups of elderly people. Some studied plasma levels of vitamin E and/or C and observed an increase after supplementation with these vitamins[9,22-26]. Some

studies also investigated the effect of a selenium supplement on glutathione peroxidase, mostly with positive results[10,23,25,26]. Only one study by Galan *et al.*[25] investigated the whole range of enzymatic and non-enzymatic antioxidants in the same study upon supplementation with a selection of vitamins and/or trace elements in hospitalized elderly people. They found increased levels of non-enzymatic antioxidants upon supplementation with vitamins and positive effects of trace element supplementation on GSH-Px levels.
We investigated whether supplementation with an enriched drink can improve biochemical status of enzymatic and non-enzymatic antioxidants in frail elderly people.

Materials and methods

Subjects. Frail elderly people aged ≥65 years were selected based on a body mass index (BMI) of less than 25 kg/m^2 and residency in a home for the elderly or sheltered housing. Subjects were not eligible for the study when they had diagnosed cancer or chronic gastrointestinal disorders (Crohn's disease, colitis ulcerosa, stoma), when they consumed diets that were not compatible with supplementation or when they were mentally unable to answer the study questions or to remember taking the supplement. The study protocol was approved by the Ethics Review Committee of Wageningen University and written informed consent was obtained before randomization.

Intervention. A randomized, double blind, placebo controlled intervention study was performed. At enrollment, subjects were stratified based on their BMI to receive a placebo or enriched drink. The enriched and placebo drinks were provided in two flavors in 125-ml tetrapacks. The drinks had to be taken in addition to the normal food consumed, two times a day, for six months. The enriched drink contained energy (100 kcal/100 ml), protein (3.5 g/100 ml of which 0.05 g cysteine), carbohydrate (11.4 g/100 ml), fat (4.5 g/100 ml), and micronutrients in amounts of approximately 30 to 150% of US RDA, with higher levels of antioxidants, ie. vitamin C (225 mg; 375% RDA), vitamin E (55 mg; 550% RDA) and selenium (85 μg;155% RDA) (Table 1). The placebo contained no energy and consisted of water, sweetener, cloudifier, thickener, flavoring and colorant. Subjects received the drinks at home during visits every two weeks. Compliance was verified every two weeks as part of home interviews and by counting leftover supplements. Compliance percentage was quantified as (number of supplements provided – number of supplements returned / (number of days participation in study * 2)) * 100%.

***Table 1**. Composition of the dietary supplement per daily dose (250 ml).*

Nutrient	
Energy (MJ) (kcal)	1.05 (250)
Protein (whey)(g)	8.75 (14 energy%)
Carbohydrates (g)	28.5 (46 energy%)
Fat (g)	11.3 (40 energy%)
Dietary fibre (g)	5.8
Sodium (mg)	78
Potassium (mg)	548
Chloride (mg)	38
Calcium (mg)	400
Phosphorus (mg)	400
Magnesium (mg)	95
Iron (mg)	9.5
Zinc (mg)	18
Copper (mg)	2
Manganese (mg)	3
Fluoride (µg)	50
Molybdenum (µg)	38
Selenium (µg)	85
Chromium (µg)	28
Iodine (µg)	88
Vitamin A (µg)	200
carotenoids (mg)	3
Vitamin D (µg)	10
Vitamin E (mg)	55
Vitamin K (µg)	50
Vitamin C (mg)	225
Vitamin B1 (mg)	2
Vitamin B2 (mg)	2
Vitamin B6 (mg)	2.5
Vitamin B12 (µg)	3.8
Niacin (mg NE)	7.5
Pantothenic acid (mg)	2.5
Folate (µg)	400
Biotin (µg)	68
Coenzyme Q10 (mg)	3
Flavonoids (mg)	19

Placebo: no energy, no vitamins, no minerals

Measurements

Anthropometry. At enrollment into the study, age and sex of participants were registered. After subjects had voided body weight was measured to the nearest 0.1 kg on a calibrated weighing scale (Seca, Germany). Knee height, as an estimate of height, was measured to the nearest 0.1 cm using a stadiometer. Body mass index (BMI) was calculated as weight/(knee height^2 * 10)[27].

Dietary intake. At baseline a three day (two weekdays and one weekend day) estimated dietary record was filled out by the participants according to the method described by de Jong *et al.*[28]. This record was checked by a dietitian. Food intake was calculated using standard portion sizes with the computerized Dutch Food Composition Table of 1997[29]. To calculate the percentage of subjects with inadequate vitamin intake, a cut-off point of 2/3 of US RDA was used (40 mg vitamin C, 6.6 mg vitamin E, 0.66 mg vitamin A and 3.3 μg vitamin D, respectively).

Blood sampling. Venous blood samples were collected in heparinized tubes at baseline and at the end of the study, in the subjects' homes after an overnight fast while they were in a sitting position. Samples were cooled immediately after collection and 1 ml of whole blood was taken and stored at –80°C until analysis of total thiol and glutathione peroxidase. The remaining sample was centrifuged within 4 hours at 2500 *g* for 10 min at 4°C. After centrifugation, the plasma layer was removed and stored at –80°C for analysis of TEAC within one month and cysteine[30] at the end of the study (24 months).

Antioxidant analysis. For vitamin E analysis 10 μl Trolox (12.5 mg/100 ml Trolox (Aldrich; 23,881-3) 25% ethanol solution) and 10 μl 0.05 mM butylated hydroxytoluene (Sigma; B-1378) were added to 0.5 ml plasma and stirred. Vitamin E was measured using a plasma ethanol dilution of 1/10 and a triple hexane extraction[31]. For vitamin C and uric acid analysis, 0.5 ml plasma was added to 0.5 ml of a solution of 10 g metaphosporic acid (Merck; 1.00546) and 15.4 mg dithiothreitol (ICN; 1000596) per 100 ml. Samples were centrifuged for 5 min at 3000 g at 4°C. Supernatant was stored at –80°C for analysis within 6 months. Vitamin C was analyzed by HPLC[32,33] and uric acid according to Margolis & Duewer[34].

Glutathione was measured within one month of sampling in whole blood as total thiol[35]. Glutathione peroxidase was analyzed according to Belsten *et al.*[36]. To correct for erythrocyte concentration, data for total thiol and glutathione peroxidase are expressed per mg hemoglobin (Hb[37]).
Plasma total antioxidant capacity (TEAC) was measured within one month of sampling[38]. TEAC was expressed as Trolox equivalent antioxidant capacity (units) which is defined as the equivalent antioxidant status of a μM concentration of a water soluble vitamin E analogue (Trolox) solution.
For all analyses the within run variation was <5%.

Statistical analysis. Normal distribution of variables was tested by the Shapiro-Wilk normality test, using Z scores. Per protocol comparisons between groups were made using Student's *t*-tests for normally distributed data and using Mann-Whitney U test for data that were not normally distributed. Two-sided *p*-values less than 0.05 were considered significant for baseline comparisons. One-sided *p*-values less than 0.05 were considered significant for comparisons of changes (6 months – baseline). All calculations were carried out using the Statistical Package SPSS for Windows, version 10.0 (SPSS Inc., Chicago, USA).

Results

Study sample. Out of the 104 included subjects, 68 completed the study period and blood samples to perform analysis of antioxidants were available for 55 participants. At baseline age, BMI, sex distribution and vitamin C level were similar for both the participants that completed the study and the dropouts (data not shown). However vitamin E levels were significantly lower in the group that completed the study (28.3 ± 8.1 vs. 33.5 ± 9.6 μmol/L; *p*=0.014). Baseline characteristics of the participants who completed the study are presented in Table 2. No significant differences were found between the two groups. Average compliance with the supplementation was 96% (range 54-130%) and did not differ between supplement and placebo group. Intake did not differ between groups, and in both groups a large proportion (14 to 80%) of subjects had calculated intakes below 2/3 of US RDA.

***Table 2**. Baseline characteristics (mean ± SD) of frail elderly participants of the nutritional supplementation study.*

	Supplement n=28	Placebo n=27	p
Male (%)	43	37	0.782
Age (years)	82 ± 7	84 ± 6	0.194
BMI (kg/m^2)	24.1 ± 2.4	23.2 ± 2.3	0.187
Energy (kJ/day)*	7595 ± 1611	7008 ± 1840	0.283
(kcal/day)	1811 ± 386	1670 ± 440	
Vitamin E (mg/day)*	8 ± 3	9 ± 4	0.694
% below 2/3 RDA (6.6 mg)	30	48	
Vitamin C (mg/day)*	65 ± 28	70 ± 45	0.642
% below 2/3 RDA (40 mg)	30	14	
Vitamin A (mg/day)*	0.5 ± 0.4	0.5 ± 0.2	0.255
% below 2/3 RDA (0.66 mg)	80	81	
Vitamin D (μg/day)*	3 ± 2	4 ± 2	0.750
% below 2/3 RDA (3.3 μg)	60	57	

* n=21 + 20

Antioxidant levels. In Table 3 the changes in non-enzymatic and enzymatic antioxidants after intervention are presented for both groups. None of the subjects had plasma vitamin E levels below normal values whereas a large proportion (about 40%) had low plasma vitamin C levels. After supplementation, no subjects in the supplemented group had plasma levels below the reference values for vitamin C, whereas in the placebo group a large proportion of deficient subjects remained.

Changes in vitamin E, vitamin C, TEAC and cysteine were significantly different between groups. There was a trend towards significance in changes in erythrocyte glutathione peroxidase. No difference was observed for uric acid and total thiol.

Discussion

In this 6-month intervention trial with an enriched drink among frail elderly people we observed a significant difference in changes in plasma vitamin E, vitamin C, TEAC and cysteine levels in favor of the supplement group.

***Table 3**. Plasma antioxidant levels (mean ± SD) and changes (mean ± SE) after 6 months supplementation in frail elderly people.*

	Supplement n=28	Placebo n=27	*p* for difference in change
Vitamin E (μmol/L)			
Start	30 ± 8	27 ± 8	
% below normal*	0	0	
6 months	46 ± 11	29 ± 9	
% below normal*	0	0	
Change	16 ± 2	2 ± 1	0.000
Vitamin C (μmol/L)			
Start	32 ± 24	33 ± 20	
% below normal*	46	37	
6 months	69 ± 23	35 ± 26	
% below normal*	0	44	
Change	37 ± 5	1 ± 5	0.000
TEAC (μmol/L Trolox eq)**			
Start	537 ± 63	549 ± 88	
6 months	575 ± 66	539 ± 101	
Change	38 ± 15	-10 ± 11	0.008
Uric acid (μmol/L)			
Start	274 ± 67	263 ± 75	
6 months	271 ± 55	267 ± 84	
Change	-3 ± 9	4 ± 9	0.514
Cysteine (μmol/L)			
Start	307 ± 57	301 ± 35	
6 months	324 ± 41	301 ± 45	
Change	17 ± 10	0.4 ± 6	0.044
Total thiol (μmol/g Hb)			
Start	9.8 ± 1.6	9.3 ± 1.6	
6 months	8.9 ± 1.4	8.9 ± 1.7	
Change	-0.9 ± 0.3	-0.4 ± 0.4	0.086
GSH-Px (U/mg Hb)			
Start	152 ± 21	159 ± 30	
6 months	151 ± 16	149 ± 14	
Change	-0.2 ± 3	-10 ± 7	0.097

* normal values: vitamin E >11.6 μmol/L, vitamin C >23 μmol/L

** Trolox equivalent antioxidant capacity, n=17+20

For our supplementation trial, we aimed to study a group of frail elderly people who are at risk of nutritional deficiencies as they will benefit most from nutritional supplementation. The intake of vitamin E in our study was below RDA for a large proportion of subjects. However, serum levels were adequate, but slightly lower than measured in healthy free living elderly people[14,39]. We observed an indication of a selection bias as with regard to serum vitamin E levels as these were lower in the group that completed the study than in the dropouts. However, no such difference was present for other parameters. Vitamin C intake was also low and plasma levels were very low, a little higher than in institutionalized elderly people[9,25] but lower than in apparently healthy free living elderly people[26,39]. From the low levels of vitamin E and C, energy intake and BMI we conclude we indeed studied a group of frail elderly people that was aimed for.

In order to improve both non-enzymatic and enzymatic antioxidant levels we used a complete supplement containing all essential vitamins and trace elements at moderate doses, with higher levels of antioxidants. The compliance with the supplement and placebo was adequate, indicated by counting the leftover packages and also by the rise in plasma levels for vitamin C and vitamin E. The antioxidants we measured are very commonly used to assess antioxidant status[40]. These have been reported to be reduced in elderly people and reflect the status of essential nutrients as provided by our supplement.

The increase in plasma levels of vitamin E and C has also been reported by other supplementation studies in elderly people living in homes for the elderly[23] or free living elderly people[22,26]. We measured TEAC as an indicator of total antioxidative capacity in the plasma. The majority of the TEAC is comprised of uric acid. Uric acid tends to increase with age, which may cover part of the antioxidant deficiency in old age[41]. As expected, but not demonstrated before, the increases in non-enzymatic antioxidant levels and the lack of change in uric acid in our study were reflected in an increase in TEAC and thus may reflect a relevant change. McKay *et al.*[26] did not find an effect of a multivitamin supplement on oxygen radical absorbance capacity (ORAC) assay. They ascribe the lack of effect to the ORAC assay and to the large contribution of uric acid. However, they did not measure the stability of uric acid over the study period. Another explanation for the lack of change is that they studied free living elderly people who had relatively high vitamin E and C levels at start and who may therefore have had only small increases upon supplementation.

Our enriched drink contained a protein source that was five times higher in cysteine (11 mg/g) than the standard casein (2 mg/g) used in nutritional supplements. This was reflected in the significant increase we found in plasma cysteine levels. Because cysteine

is an important precursor for glutathione formation, we had also expected to find a rise in total thiol levels. A study in glutathione depleted patients showed that supplementation with a whey based (cystein rich) diet increased plasma glutathione whereas a casein based diet did not[42]. Michels *et al.*[43] found similar results in HIV patients. We did not observe this effect on glutathione with our cysteine rich drink. This may be explained by the fact that the measurement of total thiol is not specific enough, as it takes into account not only glutathione but also other sulfur compounds present in the blood. Another explanation could be that the rise in cysteine was too small to affect glutathione formation.

The difference between groups we found in GSH-Px levels should largely be ascribed to the decrease in the placebo group. This can be confirmed by studies that have shown that GSH-Px levels decrease with aging[13,15,16] or with impaired health status[17]. Supplementation may have prevented a decline in GSH-Px levels in these frail elderly people over a 6-month time period. We did not measure selenium status in this study. If selenium status was adequate at the start of the study period, then this might explain the lack of increase of GSH-Px levels in the supplement group. However, Clausen *et al.*[23] reported an increase in GSH-Px after supplementation with high doses of selenium, vitamin C and vitamin E in elderly people living in homes for the elderly with an adequate selenium status. In contrast, McKay *et al.*[26] found in free living elderly people no effect of multivitamin supplementation on GSH-Px; plasma selenium levels were not measured. Thus other factors besides selenium status may influence the response of GSH-Px to supplementation.

Since our study was focused on the biochemical status of antioxidants it is important to note that the health consequences of the rise in antioxidant blood levels in our study remain unknown. Positive effects of raising antioxidant status on immune function[44] and memory[45] have been reported in the literature, and we have found similar effects in our study[46]. However, due to the nature of our supplement we cannot attribute these effects solely to the biochemical effects on antioxidant levels we observed.

We conclude that supplementation with an enriched drink with antioxidants can raise plasma levels of enzymatic and non-enzymatic antioxidants in frail elderly people.

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7

The effect of supplementation with an enriched drink on indices of immune function in frail elderly.

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Abstract

We evaluated the effect of an enriched drink on immune function in the elderly. 33 frail elderly subjects (aged ≥ 65 years and body mass index ≤ 25) received two 125 ml packages of either an enriched drink (n=20) or placebo (n=13) daily for 6 months. The enriched drink contained macro- and micronutrients. At baseline and after 6 months blood samples were drawn and PBMC's were isolated. ConA stimulated proliferation and IL-2 production of PBMC's were measured. There was a significant difference between groups in proliferation over the study period. The supplement group remained stable whereas the placebo group showed a reduction in proliferation over the 6-month period. There was no significant difference in IL-2 production between groups. Our study adds to

Submitted

the evidence that nutritional supplementation can affect immune function in the elderly.

Introduction

Ageing induces a change in immune responses[1,2]. The clinical manifestation of such decreased immune function is an increase in the susceptibility to infections and in the incidence of some autoimmune diseases and certain cancers in the elderly population[3].

Age-related functional decrease of the immune system is mainly related to a decline in T-cell mediated immunity. This is a multifactorial phenomenon affecting the number of T-cells, T-cell subset composition, and biological functions including lymphocyte proliferation and cytokine production[4]. A frequently observed phenomenon with ageing is dysregulation of the production of several cytokines. Interleukin 2 (IL-2) has been studied most extensively and a decline in IL-2 production with ageing has been observed[2,5].

Besides ageing, nutritional status is considered to have a critical influence on immune function[6,7]. Poor nutritional status results in a decrease of cell-mediated immune response resulting in a concomitant higher risk of infection and other disease[7]. Alterations in cell-mediated functions due to poor nutritional status include inhibition of delayed type hypersensitivity, impaired responses of lymphocytes to mitogens, decreased levels of IL-2 synthesis, and poor responses to inflammatory reactions[8].

Low level of food intake is prevalent in certain groups of elderly, with the highest prevalence in elderly nursing home residents[9]. However, also subjects from the SENECA study, who were free-living but had a sedentary lifestyle, had a lower energy intake than active free-living elderly. This resulted in a prevalence of inadequate intake of vitamin B1, vitamin B2, vitamin B6 and vitamin C of 61%, 31%, 58% and 18%, respectively[9]. Dietary antioxidants such as vitamins E, A and C are particularly likely to play an important role in normal immune function by protecting components of the immune cell membrane from oxidation by free radicals[10]. Other nutrients that have been related to immune function in elderly are zinc[11,12] and vitamin B6[13].

Several studies have indicated that improving nutritional status through micronutrient[12,14,15] or macronutrient supplementation[8] have positive effects on immune function in an elderly population. Therefore we chose to study effects of an enriched drink containing a combination of macro- and micronutrients.

We investigated whether provision of an enriched drink can improve *ex vivo* indicators of immune function in free-living frail elderly people.

Materials and methods

Subjects. We performed a randomised, placebo controlled parallel group intervention study. We selected frail elderly people aged ≥65 years, based on a body mass index (BMI) of less than 25 kg/m^2 and residency in a home for the elderly or sheltered housing. Subjects were not eligible for the study when they had diagnosed cancer or chronic gastrointestinal disorders (Crohn's disease, colitis ulcerosa, stoma), when they consumed diets that were not compatible with supplementation or when they were mentally unable to answer study questions or to remember taking the supplement.
The study protocol was approved by the Ethics Review Committee of Wageningen University and written informed consent was obtained.

Intervention. At enrolment, subjects were stratified based on their BMI to receive a placebo product or nutrient supplementation. The dietary supplement and the placebo were provided in two flavours in 125-ml tetrapacks. The drinks had to be taken in addition to the normal food consumed, two times a day for six months. The supplement was an enriched drink, containing energy (100 kcal/100 ml), protein (3.5 g/100 ml), carbohydrate (11.4 g/100 ml), fat (4.5 g/100 ml), and micronutrients in amounts of approximately 30 to 150% of US RDA, with higher levels of antioxidants per daily dose. The placebo consisted of water, sweetener, cloudifier, thickener, flavouring and colorant. The composition of the supplement is presented in Table I. Subjects received the drinks at home during visits every two weeks. Compliance was verified every two weeks as part of home interviews and by counting leftover supplements.

Measurements. Measurements were performed at start and after 6 months of intervention. Fasting venous peripheral blood samples (20 ml) were collected for biochemical and immunological analyses. Subjects kept a diary in which their family doctor reported any illness when asked for medical assistance. In addition, self-reported occurrence of illness was also registered every 2 weeks during a visit of the investigator. Illness was classified into infectious and non-infectious illness.

Anthropometry. At their enrolment into the study, age and sex of participants were registered. Body weight was measured to the nearest 0.1 kg after subjects had visited the

***Table I**. Composition of the dietary supplement per daily dose (250 ml).*

Nutrient	Amount per 250 ml
Energy (MJ) (kcal)	1.05 (250)
Protein (whey)(g)	8.8 (14 energy%)
Carbohydrates (g)	28.5 (46 energy%)
Fat (g)	11.2 (40 energy%)
Dietary fibre (g)	4.5
Na (mg)	80
K (mg)	550
Cl (mg)	40
Ca (mg)	400
P (mg)	400
Mg (mg)	100
Fe (mg)	9
Zn (mg)	18
Cu (mg)	3
Mn (mg)	4
F (mg)	0.8
Mo (µg)	40
Se (µg)	85
Cr (µg)	35
I (µg)	150
Vitamin A (µg)	240
Total carotenoids (mg)	3
Vitamin D (µg)	13
Vitamin E (mg)	70
Vitamin K (µg)	80
Vitamin C (mg)	250
Vitamin B1 (mg)	1.9
Vitamin B2 (mg)	1.9
Vitamin B6 (mg)	2.5
Vitamin B12 (µg)	5.3
Niacin (mg NE)	14
Pantothenic acid (mg)	4.5
Folate (µg)	480
Biotin (µg)	70
Coenzyme Q10 (mg)	3
Total flavonoids (mg)	19

toilet using a calibrated weighing scale (Seca, Germany). Knee height as an estimate of height was measured to the nearest 0.1 cm using a stadiometer. BMI was calculated as weight/(knee height^2*10)[16].

Biochemical parameters. Blood samples were centrifuged for 10 minutes at 3500 rpm and supernatant was stored at –80°C until analysis. C-reactive protein was analysed by Synchron Clinical System CX_4CE using kit 445855 (Beckman Instruments, USA) by Analytical Biochemical Laboratory (Assen, The Netherlands). Vitamin E was measured by the method

of Stump[17] using plasma ethanol dilution of 1/10 and a triple hexane extraction. Glutathione was measured as total thiol as described by Beutler *et al.*[18]. Vitamin C was analysed by HPLC according to the methods of Liau *et al*.[19] and Motchnik *et al.*[20].

T-cell proliferation and IL-2 production. Peripheral blood mononuclear cells were isolated from blood by Ficoll-Paque (Pharmacia Biotech, Sweden) density gradient centrifugation. Interface cells were collected and washed twice with sterile phosphate-buffered saline (Gibco, BRL). Cells were resuspended in complete culture medium (RPMI-1640, Gibco, BRL), supplemented with 10% fetal calf serum (FCS) and 1% penicillin + streptomycin (PIS; Gibco, BRL) and counted with a Coulter z2 cell counter. Cells were incubated on ice for 30 minutes and the medium was supplemented further to reach final concentrations of 25% FCS and 12.5% dimethylsulfoxide (DMSO, Merck KgaA, Germany). Overnight cells were frozen slowly to -80°C and stored in liquid N_2 (-196°C) until analysis.

The mitogen Concanavalin A (ConA) (Boehringer Mannheim, Germany) was used for *ex vivo* proliferation of lymphocytes. Samples containing pre- and post- intervention cells were removed from the liquid N_2 simultaneously and thawed quickly at 37°C. DMSO was removed by washing twice with culture medium. After this the pellets were resuspended in culture medium. The cell suspensions were adjusted to 100.000 cells/100 µl and incubated in triplicate in flat-bottomed microtitre plates. Each well received 100 µl of cell suspension and 100 µl of culture medium or ConA. The wells had a final ConA concentration of 0, 3 and 10 µg/ml. Cultures were incubated for 2 days at 37°C in a humidified atmosphere of 5% CO_2. A colorimetric 5-bromo-2'-deoxyuridine immunoassay kit (Boehringer Mannheim, Germany) was used for the quantification of cell proliferation after which optical density was measured.

For the quantification of cytokine secretion, cell-free supernatants from the cultures stimulated by ConA were collected and stored at -20°C until analysis. IL-2 production was measured by a sandwich enzyme linked immuno sorbent assay. IL-2 absorption values below the lowest standard value were defined as 7.4 pg/ml, the lowest standard value. Without stimulation with ConA 85% of samples gave a readout below the lowest standard value and these data are thus not reported.

To reduce inter-assay variation, pre- and post- intervention cells of each individual were measured in the same run.

Study flow chart. A flow chart of participating subjects and dropouts is presented in figure 1. Out of the 101 included participants, 68 completed the study period. Sufficient cell counts to perform analysis of immunological parameters were present from 33 participants. Age, baseline BMI, and sex distribution were similar for the group that completed the study period and had sufficient cell counts and the group that did not (data not shown). Data are thus reported only for the subjects who completed the study period and had sufficient blood counts to perform the immunological analyses.

Statistical analysis. Normal distribution of variables was tested by the Shapiro-Wilk normality test, using Z scores. Baseline comparisons between groups were made using Student's *t*-tests for normally distributed data and by Mann-Whitney U test for data that were not normally distributed. Two-sided p-values less than 0.05 were considered significant.
Logarithmic transformations were used to improve normality. Comparisons of changes over time were made using paired Student's *t*-tests within groups and Student's *t*-tests between groups. One-sided p-values less than 0.05 were considered significant as our hypothesis was that supplementation improved immune function. Incidence of intercurrent illness was tested by Pearson's Chi square. All calculations were carried out using the Statistical Package SPSS for Windows, version 10.0 (SPSS Inc., Chicago, USA). A power calculation with α=0.05 and β=0.2 was made based on the data by Chandra[15]. This resulted in a required sample size of 14 subjects for proliferation and 20 subjects for IL-2 production.

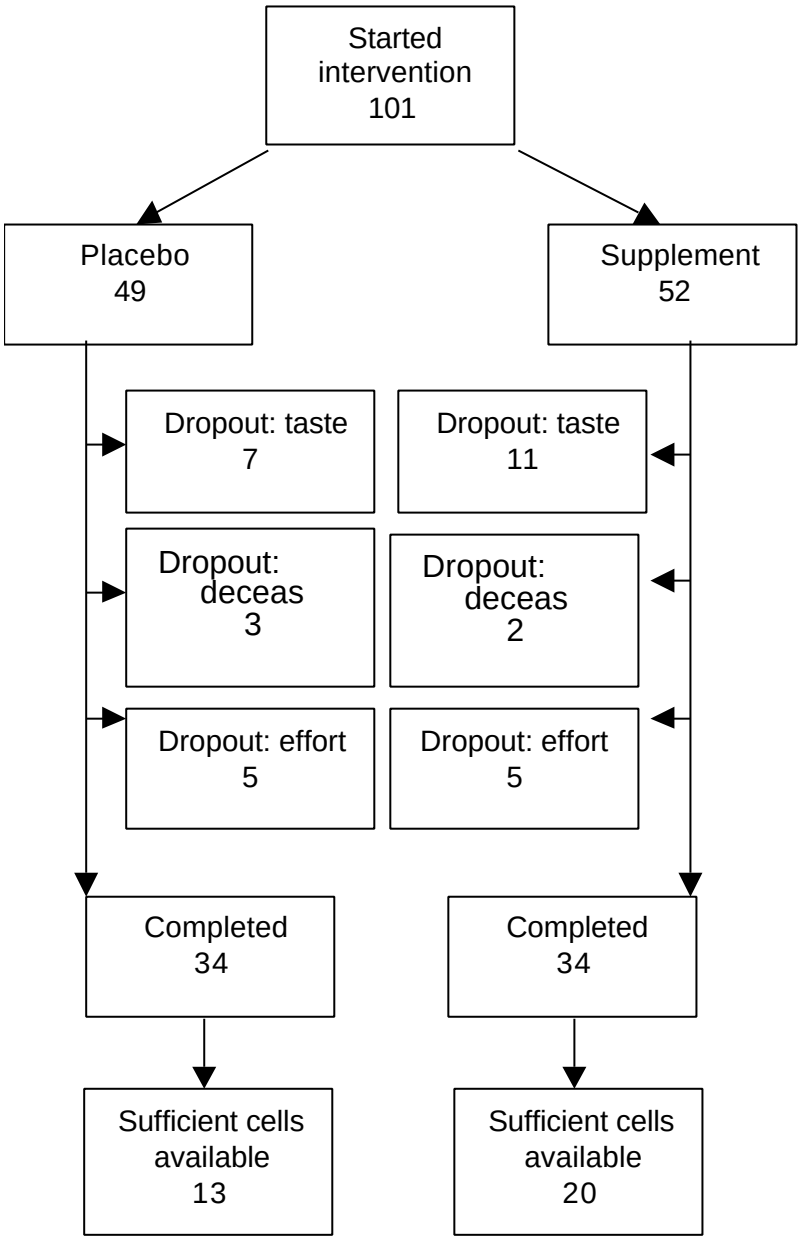


Figure 1. *Flow chart of study participants.*

Results

Study sample. Baseline characteristics of the participants who completed the study are presented in Table II. No significant differences were found between the two groups for baseline parameters except for age, which was significantly higher in the supplement group. Average intake of the supplement was 91% (range 60-100%) and did not differ between supplement and placebo group. The incidence of total illness was not significantly different for

***Table II**. Baseline characteristics of frail elderly study participants*

(Mean values and standard deviations or frequency (%))

	Placebo group (n=13)		Supplement group (n=20)	
	mean	SD	mean	SD
Male *(%)*	54		45	
Age *(years)*	81	7	86*	6
BMI *(kg/m^2)*	24.2	2.0	24.4	2.6
Vitamin C *(mM)*	33.5	22.0	31.3	25.5
α-tocopherol *(mM)*	25.9	8.7	29.1	7.4
GSH (*mM)*	1.5	0.2	1.4	0.2
CRP (mg/l)	14.0	18.4	14.1	22.8

* p=0.048

the supplemented group compared to the placebo group (53 vs. 62%, p=0.618) nor was infectious illness (40% vs. 54%, p=0.435).

Cellular immune functions

T-cell proliferation. In Figure II the changes in T-cell proliferation after intervention are presented for both groups. In the supplemented group, T-cell proliferation was similar at start and 6 months (–4% (p=0.115) for 0 μg/ml ConA, -13% (p=0.285) for 3 μg/ml ConA and -2% (p=0.795) for ConA concentration of 10 μg/ml). In the placebo group, we observed a tendency for a decline in T-cell proliferation over the study period (–8% (p=0.083), -49% (p=0.013) and –33% (p=0.021) for 0 μg/ml, 3 μg/ml and 10 μg/ml ConA respectively).

There was no significant difference in the change of T-cell proliferation after lack of mitogenic stimulation (0 μg/ml ConA) between the two groups (p=0.286). Difference in response in the

supplemented group compared to the placebo group was significant for a concentration of ConA of 3 μg/ml (p=0.008) and 10 μg/ml (p=0.012).

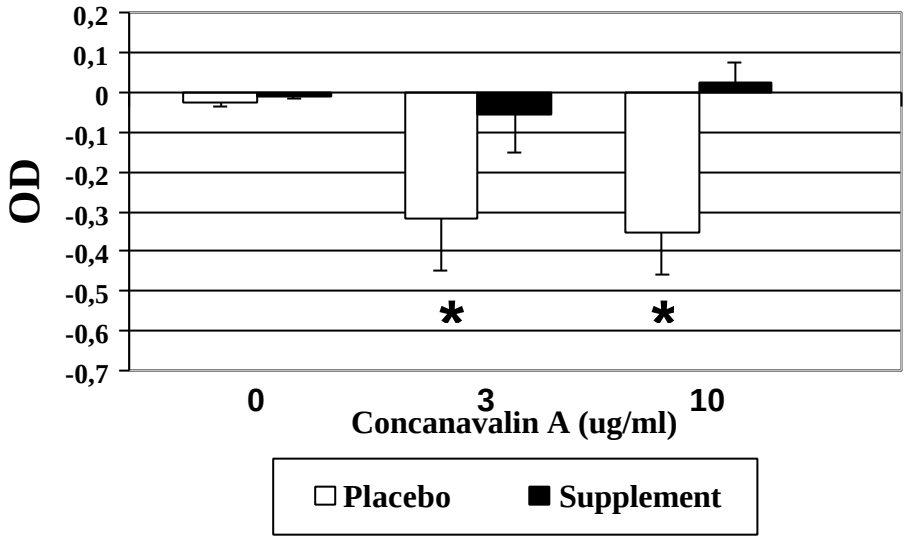
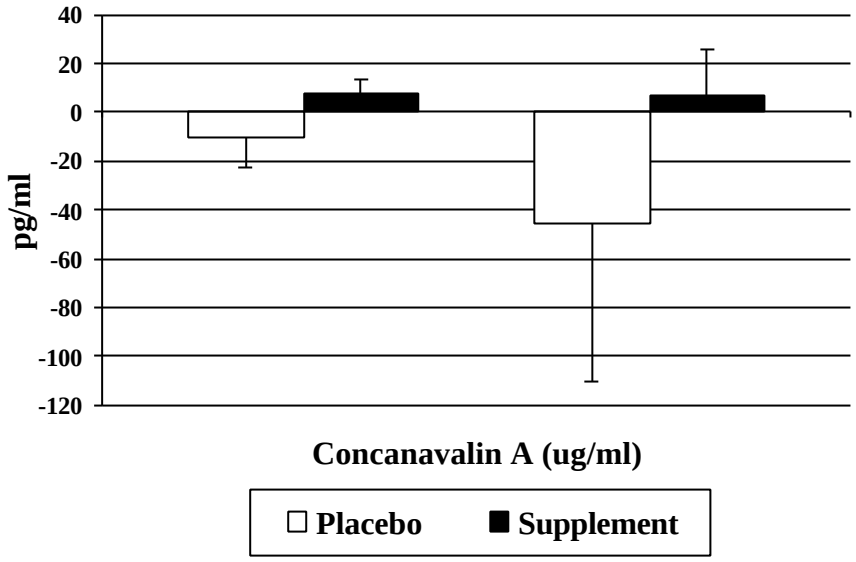


***Figure II**. Changes in T-cell proliferation (mean ± SEM) after Concanavalin A stimulation of T-cells in elderly people for placebo and supplement group (* p < 0.05 for changes between groups).*

Interleukin 2 production. For all subjects, levels without ConA stimulation were below detection limit and are therefore not reported. In the supplemented group, IL-2 production did not change significantly from start to 6 months for stimulation with 3 μg/ml ConA (23%; p=0.147) and with a ConA concentration of 10 μg/ml (14%; p=0.702). In the placebo group, IL-2 production did not change significantly after intervention for the 3 μg/ml (–16%; p=0.415) and the 10 μg/ml concentration ConA (+10%; p=0.497). In Figure III the changes in IL-2 production after intervention are presented for both groups. There were no significant differences in changes in IL-2 production between the groups for both concentrations of mitogen used (p=0.094 and 0.730 respectively for 3 and 10 μg/ml).



Discussion

In this 6-month intervention trial with an enriched drink among frail elderly we observed a significant difference in changes in proliferative response of T-cells to *ex vivo* stimulation with ConA, in favour of the supplement group. A decline in proliferative response of T-cells to ConA was observed over the study period in the placebo group. Changes in IL-2 production over the study period were not significantly different between groups.

Despite the fact that sufficient cells were available only from 33 out of 101 subjects that completed the study, it is unlikely that our results are subject to major bias because of selective dropout of subjects during the study period as baseline characteristics were not different for subjects who had sufficient cell counts and completed the study period and those who did not. The dropout rate during the study period was identical in the supplement and

Figure III. *Changes in IL-2 production (mean ± SEM) after Concanavalin A stimulation of T-cells in elderly people for placebo and supplement group.*

placebo groups. Of the subjects who completed the study period, a lower proportion in the placebo group had sufficient cells available (13/34) than in the supplement group (20/34). It may be that the number of cells available is also a measure of immune function or nutritional status. However, our study was not designed to study this as a variable and was too small to quantify such a statement. Although not significant, nutritional status defined by baseline values of vitamin E and C of the subjects of whom blood samples were studied in the laboratory seemed to be slightly better compared to the total group that completed the study period, which indicates a potential selection towards a study population with better nutritional status. We intended to select frail elderly by excluding subjects with a BMI>25 kg/m^2 from the study and recruiting only in assisted living residencies. Despite these criteria, selection bias may occur when recruiting elderly people. On the one hand, it is possible that the more active, less frail elderly consent to participate in the study and will consume the study product. On the other hand, some respondents indicated themselves to be frail and to have low nutrient intakes and therefore wanted to participate in the study.

Instead of an expected maintenance, a decline in proliferative response to *ex vivo* mitogen stimulation could be seen over the study period in the placebo group. The study was conducted from May/July until November/December, making seasonal variations a possible explanation for the decline. Adrenocortical activity varies on a circannual basis with high secretion in winter and low secretion in summer. Because adrenal corticosteroids depress cellular immune functions, a winter depression and a summer peak in T-cell function was seen by MacMurray *et al.*[21]. A study by Nieman *et al.*[22] among elderly women noted a 35% decrease in natural cell-mediated cytotoxicity in the autumn months. Haus & Smolensky[23] described seasonal variations in the response of lymphocytes to transformation. The peak in response was found during summer and the trough during winter.

Although a clear seasonal pattern in immune function and diseases is reported, changes in proliferation and IL-2 production in elderly supplementation studies are seldomly ascribed to seasonal patterns. Circannual variations are often invisible in these studies due to the duration of intervention which is often only a few weeks. Another possibility to exclude such variation would be a study period of exactly 12 months, which seems less practical in view of the already rather high dropout rate from our 6-month study.

Cell populations used for *ex vivo* mitogen stimulation, should have high viability and contain an appropriate cell population for the response being tested. In our study, viability of cells

could not be measured which can lead to underestimation of proliferative responses. The cell population was determined and counted with a Coulter cell counter, after which 1×10^5 PBMC's/100 µl were added to the plates. These amounts are similar to quantities used in previous studies in which positive effects on proliferative rate are found[13,24]. The composition of the culture medium, and the selection of mitogen is important. There can be considerable variability in individual responses to particular activating agents. In addition, the level of mitogen used becomes important in the interpretation of proliferative responses. In our study, the concentration of mitogen with the highest change in response was 3 µg/ml. Pallast *et al.*[25] found a similar amount, while others suppose 10[24] or 50 µg/ml[26] to be optimal. The culture medium and mitogen used in this study are both widely used substances known to give positive results for T-cell activation. A great variability inherent to the mitogen proliferation assays has been mentioned by Meydani *et al*.[24].

We studied IL-2 production, because a decline with ageing has been observed[5]. IL-2 is a cytokine produced by T-cells and is needed for a proper proliferation of these cells. Thus IL-2 production is inherent to T-cell proliferation[27]. The fact that we did find positive effects of supplementation on T-cell proliferation, but not on IL-2 production is potentially due to insufficient study power, although we expected to be able to observe effects based on the study by Chandra[15].

T-cell proliferation and IL-2 production have been reported as outcome measurements in supplementation studies among institutionalised elderly[14,28,29] and only a few studies have been performed in free living elderly people[15,24,30]. In addition, the type and quantity of the supplements used in these studies have varied as well as the study duration. Chandra[15] found similar results to our study, using a micronutrient supplement comparable with our levels. They found an improvement of T-cell proliferation and IL-2 production after 12 months of supplementation among a group of 96 free living elderly subjects.

In an intervention study among 32 free-living healthy older adults with a daily dose of vitamin E (800 mg) for a period of only 30 days, Meydani *et al.*[24] observed an increase in frequency and size of positive delayed type hypersensitivity responses. Proliferative response of polymorphonuclear cells to ConA (but not to phytohaemagglutinin) increased by 16%. The percent change in IL-2 concentration in the vitamin E-supplemented group (67 ± 24%) was significantly ($p<0.025$) higher than that in the placebo group (-7 ± 20%).

De Waart *et al*.[30] performed a 3-month supplementation study to assess the effect of a much lower dose of vitamin E (100 mg) on T-cell proliferation in healthy free living elderly. Vitamin E had no effect on mitogenic stimulation of cells by ConA and phytohaemagglutinin in this study.

In the above studies, micronutrients have been studied on their possible beneficial effect on immunocompetence in the elderly. However, macronutrients may also be correlated with immune responses and positive effects of protein on immunocompetence have been found[8]. This emphasises the relevance of a nutrient-complete drink as studied in our trial.

To what extent *ex vivo* results of the supplement on cell mediated immunity are related to *in vivo* responses, is still unclear. The amount of cells present in the blood of elderly people is regarded in the literature as a major contributor of effectiveness of increased proliferation *ex vivo*. Unfortunately, amounts of T-cells could not be measured in this study. Determining viability and amount of T-cells would have been useful to establish the impact of improved proliferative response in the total body. However, to perform such a study, more cells are required for which substantially more blood would need to be drawn. As studies in elderly are difficult to perform and there is a limit to the amount of blood subjects can be asked to donate, in future studies the use of new micro assay methods is needed to obtain more detailed insights into the effects of nutritional supplementation on immune function in the elderly.

Our study adds to the evidence that nutritional supplementation can affect immune function in the elderly.

Acknowledgments

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8

Effect of a complete nutritional supplement on antibody response to influenza vaccine in the elderly.

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Abstract

Background: The impact of influenza infection on morbidity and mortality in the elderly population can be severe. Influenza vaccination is not very effective in this age group which is potentially related to impaired nutritional status. We investigated the effect of a 7 months nutritional supplementation on antibody response to influenza vaccine in the elderly.

Methods: Nineteen subjects aged ≥65 years and body mass index ≤25 kg/m^2 were studied. Subjects received a complete liquid nutrition supplement containing energy, vitamins, and minerals including enhanced levels of antioxidants or non-caloric placebo drink for 7 months. Antibody titers to influenza strains A/Sydney/5/97 (SY), A/Beijing/262/95 (BE) and B/Yamanashi/166/98 (YA), before and 28 days after vaccination were measured. Age, sex, weight, height, serum albumin, serum prealbumin, hemoglobin and serum vitamin E at baseline were registered.

Results: Mean fold increase upon vaccination for SY was significantly larger in the supplement group (2.76 ± 0.66) compared to the placebo group (1.91 ± 0.66). These differences were not observed for YA (1.73 ± 0.31 vs. 1.19 ± 0.18) and BE (4.40 ± 2.63 vs. 5.76 ± 3.34). For all three strains there was no significant difference between groups in protective antibody levels (HI titer ≥40) after vaccination.

Conclusions: We conclude that provision of a complete liquid nutrition supplement including enhanced levels of antioxidants may have a beneficial effect on antibody response to influenza

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vaccination in the elderly. Further confirmation of these findings and their clinical consequences should be subject of a larger study.

Introduction

The impact of influenza infection on morbidity and mortality in the elderly population can be severe. Therefore vaccination of elderly (and other risk groups) is recommended. However, influenza vaccination has limited effectiveness in this age group (reviewed by Webster[1]) due to the fact that vaccination not always results in protective serum antibody titers. An explanation for this is that with aging there is a decreased function of the immune system, mainly related to a decline in T-cell mediated immunity. This is a multifactorial phenomenon affecting the number of T-cells, T-cell subset composition, and biological functions including lymphocyte proliferation and cytokine production[2]. A strong correlation exists between the serum hemagglutination inhibiting (HI) antibody titers to influenza viruses and clinical protection against infection[3]. In healthy well-nourished elderly only a small decline in immune function with aging is observed[4] which may mean that impaired immune function in the elderly is potentially related to comorbidity and/or impaired nutritional status. Therefore it may be possible to improve the antibody response after influenza vaccination by nutritional intervention, and reduce influenza-related morbidity and mortality.

The effect of a variety of nutritional supplements on antibody response to influenza vaccine has previously been investigated[5–10]. Supplements contained either vitamins[7], minerals[9] or both[5,6,10] or were in the form of a complete liquid nutrition supplement[8]. Most studies were performed either in elderly living in nursing homes or long stay hospital wards[7-10]. Only two studies have been performed in non-institutionalized elderly[5,6]. In some studies positive effects were found[5,6,8,10] whereas other studies did not reveal effects on antibody response to influenza vaccination[7,9]. It is of interest that in the studies describing positive effects on the antibody response a combination of nutrients was used. It seems that a combination of vitamins and minerals or a complete liquid nutrition supplement has the highest potential to improve the antibody response to influenza vaccination.

We therefore investigated the effect of a 7 months nutritional supplementation with a liquid nutrition supplement with enhanced levels of antioxidants on the HI antibody response to influenza vaccine in residents of homes for elderly people.

Methods

This randomized, double blind, placebo controlled study was part of a larger study performed from May-November 1999. Subjects aged ≥65 years with BMI (Body Mass Index:

weight/height^2) ≤25 were eligible to participate. 19 elderly of this larger study (10 supplement, 9 placebo) could be included in the substudy as they consented to both influenza vaccination and blood draw and had adequate compliance with the supplement. The study was approved by the institutional review board of Wageningen University and all subjects gave informed consent.

Subjects received nutritional supplementation with a supplement containing between 30 and 160% of the United States recommended daily allowance (RDA) of vitamins, minerals, with enhanced levels of antioxidants and 250 kcal energy twice daily for 7 months. The supplement included per 100 ml 100 kcal (0.42 MJ), 3.5 g protein, 4.5 g fat, 11.4 g carbohydrates, 1.8 g fiber, 32 mg Na, 220 mg K, 16 mg Cl, 160 mg Ca, 160 mg P, 40 mg Mg, 3.6 mg Fe, 7.2 mg Zn, 1.2 mg Cu, 1.6 mg Mn, 0.3 mg F, 16 µg Mo, 34 µg Se, 14 µg Cr, 60 µg I, 96 µg RE vitamin A, 1.2 mg carotenoids, 100 mg vitamin C, 5.2 µg vitamin D, 28 mg-α-TE vitamin E, 32 µg vitamin K, 0.75 mg vitamin B1, 0.75 mg vitamin B2, 5.6 mg NE niacin, 1.8 mg pantothenic acid, 1 mg vitamin B6, 192 µg folic acid, 2.1 µg vitamin B12, 28 µg biotin, 1.2 mg coenzyme Q10 and 7.6 mg flavonoids. Influenza vaccine (Influvac®99 containing vaccine strains A/Sydney/5/97(H3N2) (SY), A/Beijing/262/95(H1N1) (BE) and B/Beijing/184/93-like (B/Yamanashi/166/98) (YA)), was administered in October, 6 months after start of the nutritional intervention. A fasting blood sample was taken after 6 months supplementation immediately prior to vaccination and one month after vaccination[11]. Antibody response to each strain was measured in serum by hemagglutination inhibition (HI) test following standard procedures using turkey erythrocytes and four HA-units of the virus[12,13] at the University Medical Center Rotterdam. A HI titer ≥40 was considered protective[14]. Mean fold increase of antibody titers was calculated as ratio of post-immunization titer to pre-immunization titer. Vaccination history of the previous three years was registered. Chronic diseases and number of medications taken were also registered. At baseline albumin and prealbumin were analyzed by Synchron Clinical System CX_4CE using kits 442765 and 445855, respectively (Beckman Instruments, USA) by Analytical Biochemical Laboratory (Assen, The Netherlands). Vitamin E was measured by the method of Stump[15] using a plasma ethanol dilution of 1/10 and a triple hexane extraction. Hemoglobin was analyzed according to Riggs[16].

Antibody titers are reported as mean (ln antibody titer). One-tailed T-test for mean changes and analysis of variance was performed using SPSS for Windows 10.0. Wilcoxon's signed rank test was applied for mean fold increase in titer after vaccination and Fishers exact test

was used for comparison of the number of protected subjects between groups.

Results

Table I reports baseline characteristics of the study participants. No differences between groups were observed in baseline parameters.

Table I*. Baseline characteristics of elderly study participants (mean ± SD)*

Age (y)	84 ± 8
% male	42
BMI* (kg/m^2)	22.3 ± 2.1
Albumin (g/l)	38 ± 4
Pre-albumin (mg/l)	0.24 ± 0.06
Hemoglobin (g/l)	189 ± 25
Vitamin E (µmol/l)	26 ± 8
No. of medications	2.8 ± 3.0
Chronic diseases (%)	
Cardiovascular	53
Joint	21
Pulmonary	16
Gastrointestinal	16
Previous vaccination (no.)	
Yes	13
No	2
Unknown	4

* Body Mass Index (weight/height^2)

Antibody titers specific for the three vaccine strains after vaccination for placebo and supplement group before and after vaccination are given in figure I. Before vaccination, titers for BE were lower in the placebo than in the supplement group (2.9 ± 1.0 vs. 3.9 ± 1.0)(p=0.055), and did not differ for SY (4.9 ± 1.5 vs. 4.4 ± 1.0)(p=0.427) and YA (5.3 ± 1.6 vs. 5.3 ± 0.8)(p=0.923). The increase in titer after vaccination was significant in the placebo group for BE (p=0.015) and not for SY (p=0.071) and YA (p=0.418), for the supplement group the increase was significant for all three strains (p=0.002, p=0.015 and p=0.013, respectively). The changes in titer were significantly different between supplement and placebo group for SY (p=0.096) and YA (p=0.068) but not for BE (p=0.760). Analysis of variance was performed on the difference in titer before and after vaccination, correcting for titer before vaccination, which resulted in a similar effect.

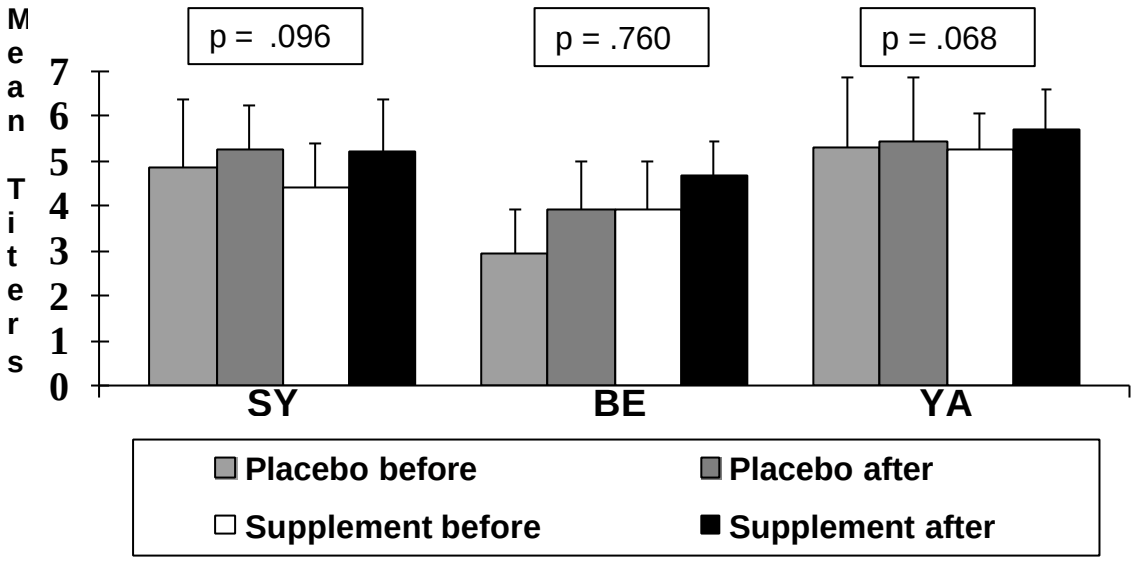
Mean Titers
0
1
2
3
4
5
6
7
p = .096
p = .760
p = .068
SY
BE
YA
Placebo before
Placebo after
Supplement before
Supplement after

Figure I*. Differences in mean titers (ln(titer) ± SD) for the influenza strains A/Sydney/5/97 (SY), A/Beijing/262/95 (BE) and B/Yamanashi/166/98 (YA) for placebo and supplement group after vaccination in elderly people (p-values for t-test of changes between groups).*

Table II reports the mean fold increase in titer post vaccination (MFI) and the number of subjects with a protective titer (≥40) before and after vaccination.

A large number of subjects had a protective titer already before vaccination. There was no significant difference in the number of protected individuals before and after vaccination between treatment groups. The mean fold increase was significantly larger in the supplement group for SY (p=0.048) but not for BE (p=0.780) and YA (p=0.091). A fourfold rise in titer, which is a criterion for an adequate effect of vaccination, could be detected only in a few subjects (SY: 1 placebo, 1 supplement; BE: 1 placebo, 1 supplement YA: 1 supplement).

Discussion

Table II*. Mean fold increase in antibody titers (MFI) and number of protected individuals (titer³40) before and after vaccination in elderly people.*

	A/Sydney/5/97 (SY)			A/Beijing/262/95 (BE)			B/Yamanashi/166/98) (YA)		
		Protected			Protected			Protected	
	MFI	Before	After	MFI	Before	After	MFI	Before	After
Placebo (n=9)	1.91 ± 0.66	8	8	5.76 ± 3.34	2	6	1.19 ± 0.18	7	8
Supplement (n=10)	2.76 ± 0.66*	6	9	4.40 ± 2.63	5	9	1.73 ± 0.31	10	10

* P<0.05 vs. placebo group

We have described increased rises in influenza vaccine induced antibody titer against two of the three influenza vaccine strains after nutritional supplementation. No significant differences in the number of protected individuals were observed between supplement and placebo group.

This report contains a per protocol analysis of subjects who did not drop out of the larger study and who had adequate compliance with the supplementation for 7 months. This may have induced a selection bias leading to exclusion of subjects who were more frail (i.e. did not feel up to blood draw or vaccination, had intercurrent illness which lead to cessation of participation in the trial). This observation is sustained by the fact that baseline characteristics of the subjects did not reveal a status of severe undernourishment. As a consequence stronger effects may have been observed if a more frail and undernourished population would have been studied. The high prevalence of adequate titers before vaccination reduced the potential for finding effects of supplementation. Explanations for this could be a study group without impaired antibody response, or the vaccine composition for the study year, containing very similar strains to previous years, which lead to high prevaccination antibody levels. The reason for the relatively small (50%) titer increases after vaccination in our study may be the fact that in the previous year a similar cocktail of strains was used. However, specifically for YA the level of protection was largely adequate before vaccination. A bias in our results due to influenza infection circulating in the community cannot be expected, as no such epidemic was reported at the time of the study.

As the influenza vaccination substudy was dependent on the season of completion of the trial, we could study only a limited number of participants. The calculated required number of subjects per group needed for significance based on the observed effect sizes was 33, 25 and 85 for SY, YA and BE, respectively.

The increase in titer rises found in our study was relatively small compared to studies with nutritional supplementation by Chandra *et al.*[5,6]. They used a supplement containing a range of micronutrients in levels comparable to our study and reported a fourfold increase in titer for a larger number of subjects in the supplemented group compared to the placebo group and an increase in geometric mean antibody titer[5]. In a later study[6] the same authors found an increase in geometric mean titer after supplementation. However, Lesourd *et al.*[8] reported an increase of about 30% in antibody titer, which is similar to our study. Mean fold increases reported by Girodon *et al.*[10] after supplementation were even smaller than in our study (an

increase of about 20%). In their study, the number of protected individuals remained low even after intervention and the number of protected individuals before vaccination was not reported. In general the data of studies on nutritional supplementation often report different calculations of titers, which complicates comparisons.

We conclude that provision of a complete liquid nutrition supplement containing enhanced levels of antioxidants may have a beneficial effect on antibody response to some influenza vaccine strains in the elderly and therefore may improve the induction of protective immunity. Further confirmation of these findings and their clinical consequences should be subject of a larger study.

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9

General discussion

The aim of the studies described in this thesis was to evaluate the effects of nutritional supplementation on several aspects of health in elderly people.

A first study of nutritional supplementation was performed to investigate whether short-term nutritional supplementation of psychogeriatric nursing home residents could prevent loss of body weight during illness (Chapter 2).

To also investigate the effect of a longer-term supplementation with a newly developed nutritional supplement on body weight, plasma vitamins and activities of daily living a second study was performed. The selected target group was a group of institutionalised psychogeriatric elderly people who were expected to be at high risk of nutritional deficiencies (Chapter 3).

The selection of psychogeriatric nursing home residents for the first two studies was based on the fact that these elderly people are well accessible for studies and in relatively stable health compared to somatic nursing home residents. However, the group of elderly psychogeriatric nursing home residents is only small, and only a limited number of outcome measures can be studied in this population, due to e.g. difficulty in understanding instructions. The alternative would be to select somatic nursing home patients, but in this population there is a large variety in medical conditions, which affects the measurement of functional parameters, and also this group forms a small fraction of the elderly population. Therefore, to evaluate the health effects of nutritional supplementation in a larger group of elderly people at risk of nutritional deficiencies, a long-term intervention study was performed in frail elderly people living in homes for the elderly or sheltered housing and having a BMI below 25 kg/m^2. This study was focused on functional outcome variables as far as measurable within the timeframe chosen (study described in Chapter 4-8).

The first part of this chapter summarises the main findings of these studies, followed by a discussion on some methodological aspects and the implications of these findings and recommendations for further research. This chapter ends with the general conclusions of this thesis.

Main findings

Our studies in the population of psychogeriatric nursing home residents (Chapter 2&3) demonstrated that both short and longer-term use of a nutritional supplement improves weight. Furthermore, plasma levels of micronutrients increased upon supplementation and the intervention was well tolerated in this target group.

Our long-term study in frail elderly people not only demonstrated weight gain (Chapter 4)

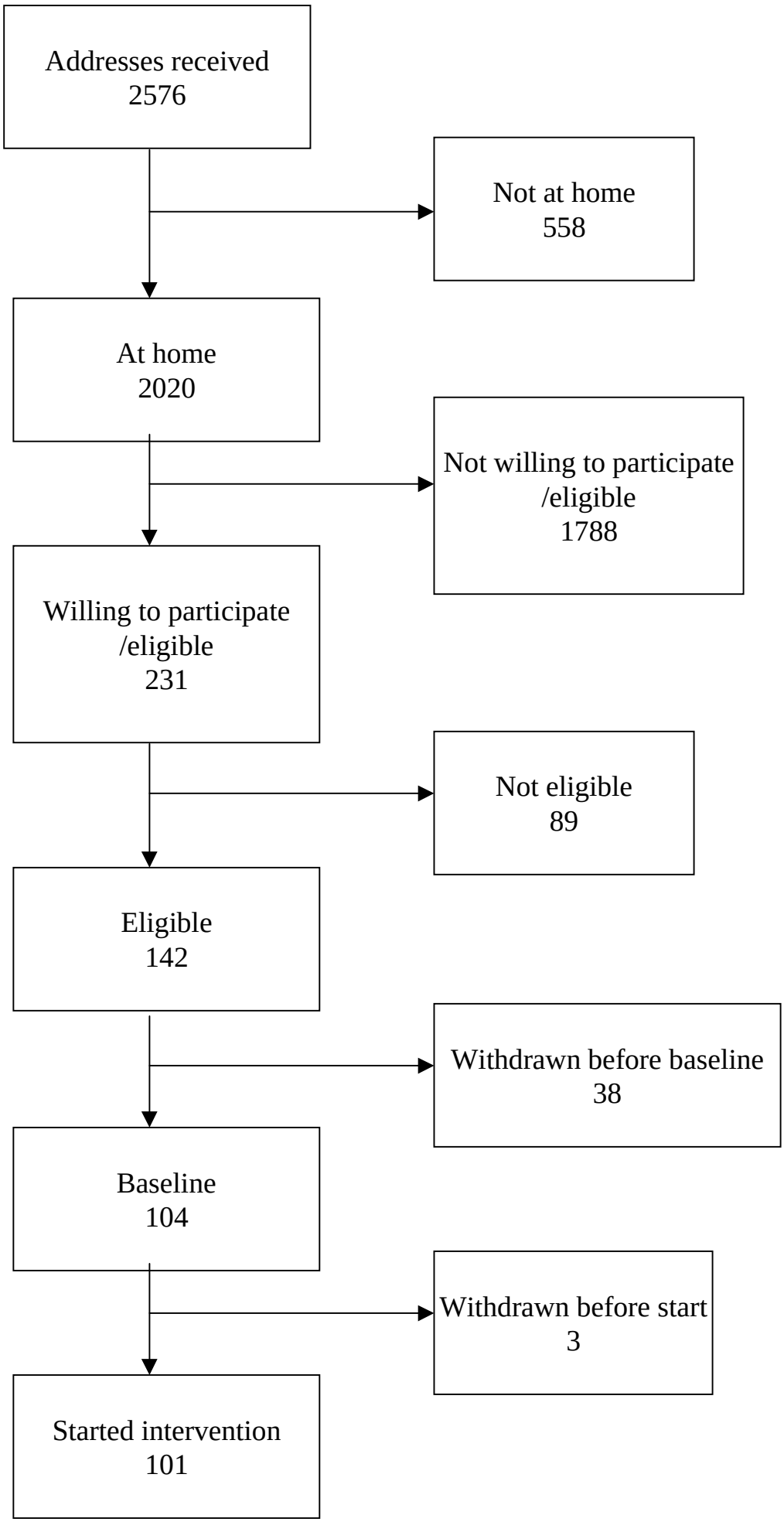
but also improvement in plasma levels of vitamins (Chapter 5&6). Plasma antioxidant (Chapter 6) and immune status (Chapter 7) improved after supplementation, and there was a trend for a better antibody response to influenza vaccine in a subgroup of this study (Chapter 8). Supplementation improved cognitive function (Chapter 5), but no major effects could be found on selected anthropometric and functional parameters or quality of life (Chapter 4).

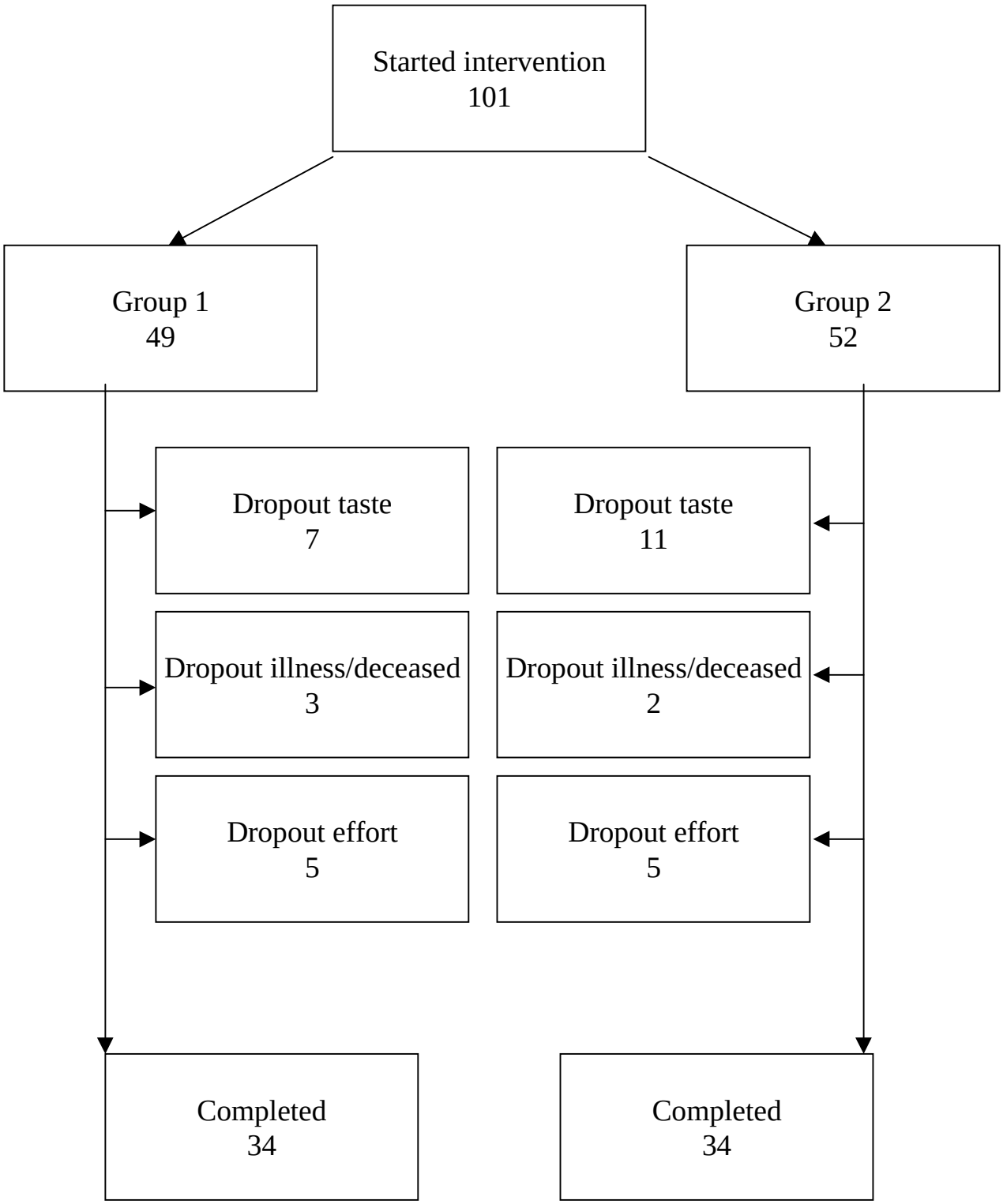
Methodological considerations

Subjects

Our first and second study selected psychogeriatric nursing home patients on several wards. As the majority of subjects who fell within the inclusion criteria participated (presence of illness in the first study and low BMI in the second study) we expect to have studied an at risk group of the nursing home population. Indeed the nutritional inadequacies observed in our second study were largely comparable to a previous study in a nursing home population[1].

For the third study, we aimed to select subjects from the population of elderly living in homes for the elderly or sheltered housing. We approached subjects by sending an information letter about the study and then visited them personally to ask for participation in the study. Figure 1 represents a scheme of recruitment. Twenty percent of subjects was not at home, and of the subjects at home, 11% was estimated eligible and willing to participate. Upon further interview, only 61% were eligible to participate in the study. Including people who had second thoughts on participation upon start of the study, we therefore had a study population representing 4% of the elderly population we approached. Of our study group 50% were living in homes for the elderly and 50% in sheltered housing. A factor in the low response was our selection criterion of BMI $\leq$25 kg/m^2, as we encountered many subjects who had a weight too high to participate. This is confirmed by numbers of the general elderly population that 49% of men and 51% of women have a BMI>25 kg/m^2 [2]. Some elderly indicated themselves either that they were "too old" to participate or "too healthy". As subjects needed to be able to take the supplements daily and remembering to do this, we assume that the population we selected is in slightly better mental condition than the average population living in homes for the elderly and sheltered housing residences. If we compare the scores of our population on several aspects of health with data available from literature on institutionalised, frail and apparently healthy elderly people (table 1), it is however clear that these scores lay


Addresses received
2576
Not at home
558
At home
2020
Not willing to participate
/eligible
1788
Willing to participate
/eligible
231
Not eligible
89
Eligible
142
Withdrawn before baseline
38
Baseline
104
Withdrawn before start
3
Started intervention
101


Started intervention
101
Group 1
49
Group 2
52
Dropout taste
7
Dropout taste
11
Dropout illness/deceased
3
Dropout illness/deceased
2
Dropout effort
5
Dropout effort
5
Completed
34
Completed
34

between healthy and institutionalised elderly people and thus we have studied the population we aimed for. In view of the fact that the longer-term study was a follow-up study with the same supplement in another target group, it may also be noted that indeed similar effects of the supplement on body weight and plasma nutrient levels were observed for both studies.

Table 1. *Characteristics of our study population and some studies of institutionalised, frail and apparently healthy elderly.*

Population	Reference	N	Age (y)	BMI (kg/m^2)	Chronic diseases (no)	Medication (no)
Nursing home	3	84	71	22	-	-
	1	33	81	-	-	6
Free living frail	4	50	88	25	5	8
	Our study	**68**	**82**	**24**	**2**	**4**
	5	159	79	24	-	2.5
Free living healthy	6	80	67	28	-	-
	7	51	74	27	0	-

- = not reported

Dropout. We observed a dropout rate of 33% of subjects who started the study. Main reason for dropout given by the subjects was the palatability of the supplements, followed by the effort it took to participate in the study and the occurrence of illness that prohibited intake of the supplement or performance of measurement of outcome parameters. The majority of dropouts occurred soon after the start of the study. Our biweekly visits were aimed at preventing dropouts, but unfortunately could not prevent these completely. Specifically there was a proportion of subjects who had not realised the implications of participation in the study. As these subjects only indicated refusal to participate after start of the study, they were to be treated as dropouts and evaluated on an intention-to-treat basis. We did perform these analyses, and outcomes showed a similar direction as the per protocol analyses. We did not report only intention-to-treat analyses in the previous chapters because we were aiming to evaluate the efficacy of the nutritional supplement and not the effectiveness of the intervention as such.

Missing values of blood sample analyses. The maximum amount we considered ethical for blood draw (20 ml) allowed only a selection of biochemical analyses to be performed. This was specifically the case for the immunological analysis of T-cells and less so for the analyses of the range of antioxidants. This may have introduced a selection bias in subjects based on the quantity of blood they could give (potentially related to the thickness of the blood) and the quantity of plasma of serum available (potentially related to the hydration status of the subject). Especially the post-hoc analysis of homocysteine and vitamin B12 could only be performed in a highly selective sample. Future development of more efficient methods for assays that require less sample material may improve the possibilities for investigating different biochemical aspects of supplementation.

Methods

Design. A good study design and execution of placebo controlled trials is aimed to decrease variability in measurements due to observer or time effects. We ruled out a large proportion of interobserver variation by having the same investigator evaluate the subjects at start and end of the study. However, for practical reasons we were not able to control the daytime of observation, which may have introduced some variability of measurements. Due to the continuous enrolment of subjects in the study, the observations took place at different times of the year. However, at any timepoint of the study an equally randomised group over supplement and placebo was studied.

Questionnaires. The use of questionnaires to measure outcome variables may not be adequate to pick up any small effects presenting over a six-month period. Many elderly subjects seemed to give socially desired answers to the questions or gave answers that were true for their past, but not for their present condition. A discrepancy between self-reported and performance based tests in the elderly has been reported[8] and related to socio-demographic and personality aspects[9]. This affected the questionnaires we used measuring activities of daily living, depression and quality of life and may explain the fact that we found little effect of supplementation on these outcomes.

Comparison to literature

Our study is unique in evaluating the effect of a “complete” supplement on several health aspects (such as body composition, memory, immune function and quality of life) in elderly people. Other studies have mostly examined different target groups, supplements and

used other (single) outcome measures.

Table 2a in the Appendix gives an overview of double-blind randomised, placebo controlled nutritional intervention studies performed in general populations of elderly people (thus not selected based on the presence of a specific disease). Several studies with nutritional supplements do not include a placebo group but only a control group, which makes effects more difficult to evaluate as this design does not take into account aspects of compliance and also subjectivity, as both investigator and subject are not blinded to the intervention. These studies are reported separately in table 2b in the Appendix. Outcome measures vary widely, but most studies report positive effects of nutritional intervention on one or more outcomes. From this table it is clear that our studies have shown that the combination of macronutrients and micronutrients affects several aspects of nutritional status and health of the groups of psychogeriatric and frail elderly.

Impact of the findings

For elderly people.

Our studies have shown that nutritional supplementation improves the nutritional status of elderly people and through this improves their physiological functioning as defined by immune function, mental performance and sleep quality. Elderly people therefore should not merely accept a functional decline over time but among other possible measures could combat this by the use of nutritional supplements. Many of the elderly in our third study considered themselves not as at risk for impaired nutritional status and its consequences. When awareness occurs that in certain risk situations, such as illness, loneliness, etc.[10], they risk obtaining an impaired nutritional status and that use of a nutritional supplement at that time may even prevent any deterioration, they may preserve quality of life.

For health care and government.

In discussions on the care for the ageing population, nutrition is often not mentioned or only as a secondary issue. However, as a good nutritional status is a prerequisite for health of the elderly, nutrition is highly relevant when considering e.g. need for medication and costs of care. As our studies have demonstrated improvement in several indicators of health in the elderly, nutritional supplementation may help reduce the need for care. This may lead to a relief of the burden of care for society in general.

Implementation

Feasibility of dietary supplementation of energy

One of the questions posed when providing a nutritional supplement is whether it is effective at all because it may lead to a decline in intake of regular foods and thus have no net results. It is true that this is the case when providing the supplement at mealtimes, but most studies have clearly shown no such compensation of intake[11,12] when served between meals. The reason for this is possibly that elderly loose the ability of physiological compensation of energy intake[13,14]. Thus the use of a supplement as we applied in our studies will have positive effects on energy and nutrient intakes.

For any functional effects that are mediated through muscle mass, provision of energy and protein is essential[15]. It has been postulated that the mere provision of micronutrients may enhance functional status, mediated through an increase in appetite. Although a mechanism for the effects of micronutrients on appetite may be their effect on depression and mood[16], to date no studies have shown an increase in appetite due to a micronutrient supplement.

It has to be noted that energy intake for a proportion of elderly is adequate or may not need to be increased as they are overweight. However, even in overweight elderly deficiencies of micronutrients exist and thus they also benefit from effects of nutritional supplementation on immune function, cognitive function and other health aspects by provision of a supplement with micronutrients only.

The provision of a nutritional supplement per se does not provide a guarantee for improving nutritional status. A study by Ross *et al.*[17] showed that not all subjects who needed a nutritional supplement actually received it and that 39% of people who did, hardly consumed it. Therefore, support of any nutritional supplementation regimen by caretakers and/or family is important.

Finally, when developing nutritional supplements for the elderly, specific attention should be paid to changes in taste preferences of the elderly. A decreased flavour perception and increase of preferred level of sweetness has been reported with ageing[18], and therefore supplements for the elderly would need to have a stronger flavour and be slightly sweeter than standard. In the development of the supplement, we paid specific attention to these issues and the supplement was well accepted by the subjects. However, individual preferences remain, and therefore preferably a range of supplement flavours should be available for the elderly.

Combination with other interventions

A good nutritional status is a prerequisite for optimal functioning of the elderly. It may however be that by combining nutritional interventions with other interventions, larger effects on outcome variables could be obtained.

Exercise

It has been postulated that exercise is very important for the well being of elderly.
Exercise can increase energy requirements[19] and through this may establish a higher energy intake with concomitant higher micronutrient intakes. A study investigating this topic specifically however could not confirm this theory[20]. To date a few studies have been performed investigating the combination of nutritional and exercise intervention. Fiatarone *et al.*[21] found no functional effect of nutritional supplementation besides exercise. A second study in frail elderly also did not show any interaction between a dietary intervention and exercise[22], but did show additive effects. This may be explained by the fact that the dietary intervention was only based on micronutrients and a rise in protein intake may be necessary for optimal effects[23]. A similar study was performed by Bunout *et al.*[7], also indicating no additive effects of nutritional supplementation and exercise, but in this study the compliance with both regimens was low.

Hormone replacement therapy

As mentioned previously, there are many physiological changes in elderly people, among which hormonal changes may be the ones with the largest impact[24]. Hormone substitution programmes have been proposed to maintain health and prevent functional decline in the elderly[24-26]. As nutritional supplementation may be more effective in an optimal metabolism, also this approach seems interesting. An example of such a combination is the effect of calcium and oestrogen treatment on bone density in women[27] and calcium, vitamin D and testosterone treatment on bone density in men[28].

Psychotherapy

The area of memory performance is influenced by psychotherapy. To date, special memory training programmes for elderly already exist, although their effectiveness remains to be studied further[29]. By participation in such programmes alongside the use nutritional supplements potentially large improvements of memory function are attained. However, until now no studies have been performed to evaluate this approach.

Future of nutritional supplementation in the elderly

The studies described in this thesis have investigated the effect of provision of a range of nutrients currently classified as essential to elderly who are at risk of a deficiency of these nutrients.

To date, still little research is done on functional bioavailability of essential nutrients and most recommendations are still based on prevention of deficiency diseases and not on optimal effects for prevention or cure of health problems. Thus restoring intake and plasma levels of essential nutrients is a first step towards improvement of health for the elderly. Our studies have shown positive effects of such supplementation, but more information should be gathered on optimal quality and quantity of nutritional supplements and their effects on health indices.

A second step for the future is to investigate whether, once balances are restored, other bioactive components can exert any additive effects (and thus not only whether they can compensate for the lack of essential nutrients). Components that have been evaluated for such effects and that may be useful for elderly supplements in the (near) future include specific fatty acids[30,31], creatine[32,33], enzymes[34], leucine[35] and other amino acids[36], probiotics[37], glucosamine[38], gingko biloba[39] and ginseng[40]. To date, little is known about their effects on functional health parameters in elderly and this remains to be investigated in the future.

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Summary

Life expectancy in both the Western and non-western population has been increasing over the past centennial, due to improved hygiene, the discovery of medicines, such as antibiotics, and economic welfare. The consequence for society of this ageing of our population is an increased need for medical and social care and thus a burden of costs for health care. At the level of individual people an increased life expectancy is appealing, but only if accompanied by a preservation of a certain health status.

Nutrition is one of the factors that play an important role in maintaining health in elderly people. Poor nutritional status has been related to both morbidity and mortality. Low intakes or plasma levels of nutrients have been related to several areas of physiological decline often observed in elderly people. Among these relations are bone density, memory, cardiovascular disease, physical functioning, immune function, lung function and eye function.

The group of elderly that has mostly been reported to have an impaired nutritional status is institutionalised elderly. In the Netherlands, 3% of elderly people aged 65-79 are living in institutions, and this number increases to 21% for age 80 years and over. The nutrient intake of Dutch institutionalised elderly has been investigated previously and vitamin deficiencies are prevalent, especially for the vitamins B1, B6, C and D.

However, also elderly living at home may be at risk for nutritional deficiencies. In this group nutrient intake is often inadequate and plasma levels of vitamin D, vitamin B6 and vitamin B12 are deficient in a large proportion.

There are many different ways to improve nutritional status, mostly aimed at improving nutrient intake. An improvement of quantity of intake, quality of intake or a combination of both may be established. Nutritional supplementation results in improvements in both quantity and quality of intake and therefore positively affects nutritional status.

To date, our knowledge about the effects of supplementation with a combination of macro- and micronutrients on functional outcomes in elderly who are at risk of poor nutritional status is limited. Most studies so far have investigated only nutritional status as outcome parameter or evaluated effects of single nutrients or single functional parameters. Taking into account all these factors, this thesis focuses on nutritional related health indicators as outcome measures.

A first study of nutritional supplementation was performed to investigate whether short-term nutritional supplementation in psychogeriatric nursing home residents could prevent loss of body weight during illness (Chapter 2). Residents received 200 ml of a nutritional supplement daily for 4 weeks after they had been diagnosed with infection. Outcome parameters were anthropometry and need for care. To investigate the effect of longer term supplementation with a newly developed nutritional supplement on body weight, plasma vitamins and activities of daily living a pilot study was performed. The selected target group was a group of institutionalised psychogeriatric elderly people who were expected to be at high risk of nutritional deficiencies. Residents received 125 ml of a complete nutritional supplement twice daily for 12 weeks (Chapter 3). These studies in the population of psychogeriatric nursing home residents demonstrated that both short and longer-term use of a nutritional supplement was well accepted and tolerated in this target group. Furthermore, body weight and plasma levels of micronutrients increased upon supplementation.

The selection of psychogeriatric nursing home residents for the first two studies was based on the fact that these elderly people are well accessible for studies and in relatively stable health compared to somatic nursing home residents. However, the group of elderly psychogeriatric nursing home residents is only small, and only a limited number of outcome measures can be studied in this population, due to e.g. difficulty in understanding instructions. Therefore, to evaluate the health effects of nutritional supplementation in a larger group of elderly people at risk of nutritional deficiencies, a long-term intervention study was performed in frail elderly people living in homes for the elderly or sheltered housing and having a BMI below 25 kg/m^2. This study was focused on functional outcome variables as far as measurable within the timeframe chosen. Subjects received a similar complete nutritional supplement as in the second study in an amount of 125 ml twice daily. The study similarly not only demonstrated weight gain (Chapter 4), but also improvement in plasma levels of vitamins C, E, B12 and homocysteine (Chapter 5&6). Plasma antioxidant (Chapter 6) and immune status (Chapter 7) improved after supplementation, and there was a trend for a better antibody response to influenza vaccine in a subgroup of this study (Chapter 8). Supplementation improved cognitive function (Chapter 5), but no major effects could be found on selected anthropometric and functional parameters or quality of life (Chapter 4). The objective for subjects to maintain intake from the regular diet was reached, and thus their total nutrient intake increased when using the supplement.

In conclusion, the provision of a nutritional supplement can improve several health indices in elderly people who are at risk of nutritional deficiencies. Therefore, the strategy to improve health of elderly people should incorporate the option of providing nutritional supplements.

Samenvatting

De levensverwachting voor zowel de Westerse als niet-Westerse populatie is over de afgelopen eeuw toegenomen, ten gevolge van verbeterde hygiëne, de ontdekking van medicijnen, zoals antibiotica, en economische welvaart. De consequentie van deze veroudering van onze populatie voor de samenleving is een toegenomen behoefte aan medische en sociale zorg, die een last vormt voor kosten van de gezondheidszorg. Op individueel niveau is een toegenomen levensverwachting aantrekkelijk, maar alleen wanneer deze vergezeld gaat van een behoud van een zekere gezondheidsstatus.

Voeding is één van de factoren die een belangrijke rol spelen in het behoud van de gezondheid van oudere mensen. Een slechte voedingsstatus is gerelateerd aan zowel morbiditeit als mortaliteit. Een lage inname en lage plasma niveaus van nutriënten zijn gerelateerd aan verschillende gebieden van fysiologische achteruitgang die vaak voorkomen bij oudere mensen. Onder deze gebieden vallen botdichtheid, geheugen, cardiovasculaire ziekten, fysiek functioneren, immuunfunctie, longfunctie en oogfunctie.
De groep ouderen waarvan het meest gerapporteerd is dat deze een verminderde voedingsstatus hebben zijn de geïnstitutionaliseerde ouderen. In Nederland woont 3% van de mensen tussen 65 en 79 jaar in instellingen en dit aantal neemt toe tot 21% voor de leeftijdsgroep van 80 jaar en ouder. De nutriëntinname van Nederlandse ouderen is in het verleden onderzocht en vitaminedeficiënties zijn vaak aanwezig, met name van de vitamines B1, B6, C en D.
Echter, ook thuiswonende ouderen kunnen kans hebben op voedingsdeficiënties. In deze groep is de voedselinname vaak inadequaat en in een groot gedeelte zijn plasma niveaus van vitamine D, vitamine B6, en vitamine B12 te laag.

Er zijn vele verschillende manieren om de voedingsstatus te verbeteren, grotendeels gericht op verbetering van de voedselinname. Een verbetering van de kwantiteit van inname, kwaliteit van inname of een combinatie daarvan kan bereikt worden. Voedingssuppletie resulteert in verbeteringen in zowel de kwantiteit als de kwaliteit van de inname en heeft daarom een positief effect op de voedingsstatus.

Op dit moment is onze kennis van de effecten van suppletie met een combinatie van macro- en micronutriënten op functionele uitkomstmaten bij ouderen, die kans hebben op een slechte voedingsstatus, beperkt. De meeste studies tot nu toe hebben ofwel alleen voedingsstatus als uitkomstmaat bestudeerd ofwel slechts de effecten van enkelvoudige nutriënten of enkelvoudige functionele parameters geëvalueerd. Al deze factoren in overweging genomen richt dit proefschrift zich op voedinggerelateerde gezondheidsindicatoren als uitkomstmaten.

Een eerste studie van voedingssuppletie is uitgevoerd om te onderzoeken of korte termijn voedingssuppletie bij psychogeriatrische verpleeghuisbewoners het verlies van lichaamsgewicht tijdens ziekte kan voorkomen (Hoofdstuk 2). Bewoners kregen dagelijks 200 ml van een voedingssupplement gedurende 4 weken nadat ze gediagnosticeerd waren met een infectie. Uitkomstparameters waren lichaamssamenstelling en zorgbehoefte. Om het effect van langere termijn suppletie met een nieuw voedingssupplement op lichaamsgewicht, plasma vitamines en activiteiten van dagelijks leven te onderzoeken is een verkennend onderzoek uitgevoerd. De geselecteerde doelgroep was een groep geïnstitutionaliseerde psychogeriatrische ouderen die naar verwachting een grote kans op voedingsdeficiënties had. Bewoners kregen tweemaal daags 125 ml van een compleet voedingssupplement gedurende 12 weken (Hoofdstuk 3). Deze studies in de populatie van psychogeriatrische verpleeghuisbewoners toonden aan dat zowel korte als lange termijn gebruik van een voedingssupplement goed geaccepteerd en getolereerd wordt in deze doelgroep. Daarnaast namen lichaamsgewicht en plasma niveaus van micronutriënten toe na suppletie.

De selectie van psychogeriatrische verpleeghuisbewoners voor de eerste twee studies was gebaseerd op het feit dat deze ouderen goed toegankelijk zijn voor studies en een relatief stabiele gezondheid hebben vergeleken met somatische verpleeghuisbewoners. Echter, de groep van oudere psychogeriatrische verpleeghuisbewoners is slechts klein, en slechts een beperkt aantal uitkomstmaten kan in deze populatie worden bestudeerd, tengevolge van bijv. problemen met het begrijpen van instructies. Om de gezondheidseffecten van voedingssuppletie in een grotere groep ouderen met kans op voedingsdeficiënties te evalueren is daarom een lange termijn interventiestudie uitgevoerd bij fragiele ouderen die in verzorgingshuizen of aanleunwoningen wonen en een body

mass index hebben lager dan 25 kg/m^2. Deze studie was gericht op functionele uitkomstmaten voor zover meetbaar in de gekozen tijdsperiode van 6 maanden. Deelnemers kregen een vergelijkbaar compleet voedingssupplement als in de tweede studie in een hoeveelheid van 125 ml tweemaal per dag. Deze studie toonde niet alleen gewichtstoename (Hoofdstuk 4), maar ook een verbetering in plasma niveaus van vitamines C, E, B12 en homocysteïne (Hoofdstuk 5&6). Plasma antioxidant niveaus (Hoofdstuk 6) en immuunstatus (Hoofdstuk 7) verbeterden na suppletie, en er was een trend voor een betere antilichaamrespons tegen het griepvaccin in een subgroep van deze studie (Hoofdstuk 8). Suppletie verbeterde cognitief functioneren (Hoofdstuk 5), maar er werden geen beduidende effecten gevonden op de geselecteerde lichaamssamenstelling en functionele parameters of de kwaliteit van leven (Hoofdstuk 4). Het doel voor deelnemers om inname van het reguliere dieet te handhaven werd bereikt, en dus nam hun totale voedingsinname toe bij gebruik van het supplement.

In conclusie, verstrekking van een voedingssupplement kan verschillende gezondheidsindicatoren van ouderen die kans hebben op voedingsdeficiënties verbeteren. Daarom zou de strategie om de gezondheid van ouderen te verbeteren de mogelijkheid van verstrekking van voedingssupplementen moeten bevatten.

Appendix

Table 2a. *Overview of double-blind randomised, placebo controlled intervention studies performed in elderly people.*

Author	Target group	N	Age (y)	BMI / *kg*	Intervention	Nutrients	Duration	Parameters	Outcome
ONLY ENERGY									
Carver, 1995 [1]	psychogeriatric hospital dementia underweight	20+20	75	18	liquid formula 200 ml oral vitamin	600 kcal	12 weeks	weight skinfolds MUAC	improved improved improved
COMPLETE SUPPLEMENT									
Wielen, 1995 [2]	nursing home patients somatic	18+15	81	*66*	fruit juice + vitamins+minerals regular fruit juice	1680 kJ/d, 50% RDA 465 kJ/d, 40 mg C	12 weeks	body weight plasma levels ADL grip strength	improved improved = =
Fiatarone, 1994, 2000 [3,4]	long term care for elderly facility	24+26	88	26	liquid supplement artificially flavoured liquid	360 kcal ½ RDA	10 weeks	dietary intake plasma levels weight physical activity	= = improved =
Wouters-Wesseling, 2002 [5]	nursing home patients psychogeriatric	16+19	82	21	liquid supplement placebo	275 kcal ¼ -1 RDA	3 months	dietary intake plasma levels weight ADL	improved improved improved =
Wouters-Wesseling, submitted	elderly homes	34+34	82	24	liquid supplement placebo	250 kcal ¼ -1 RDA	6 months	plasma levels dietary intake body weight immune function quality of life ADL grip strength cognition	improved improved improved improved = = = improved
Yamaguchi, 1998 [6]	free living meals on wheels	30+32	78	24	liquid supplement fruit-flavoured beverage	300 kcal 105 kcal	18 months	dietary intake body weight	improved =
MULTI NUTRIENT SUPPLEMENT									
Asciutti-Moura, 1993 [7]	nursing home	41+43	71	22	E, C, B1, B2, B6, niacin, Ca placebo	15 IU, 200 mg, 7.5 mg, 9 mg, 11 mg, 35 mg, 15 mg	30 days	plasma levels	improved
Suboticanec, 1989 [8]	elderly homes	50+50	-	26	A, E, B1, B2, niacin, B6, B12, folate, B12, C placebo	5000 IU, 20 IU, 3 mg, 3.5 mg, 20 mg, 4.5 mg, 15 mg, 0.8 mg, 6 µg, 150 mg	8 weeks	plasma levels grip strength DTH	improved = improved
Clausen, 1989 [9]	homes for old people	43+54	75	-	Se, Zn, C, A, B6, E, GLA Placebo	300 ug, 45 mg, 270 mg, 2.7 mg, 6 mg, 465 mg, 250 mg	12 months	plasma levels antioxidants cognition	improved improved improved
Galan, 1997 [10,11]	nursing home patients	127+ 123+ 132+ 123	84	24	zinc + selenium vitamin C, beta carotene, vitamin E min+vit placebo: Ca	20 mg+100 µg 120 mg+6 mg+15 mg	2 years	plasma levels DTH influenza morbidity mortality	improved = improved improved =

Author	Target group	N	Age (y)	BMI / *kg*	Intervention	Nutrients	Duration	Parameters	Outcome
Penn, 1991 [12]	hospital long stay	15+15	84	*56*	vitamin C, A, E placebo	100 mg, 8000 IU, 50 mg	28 days	plasma levels T-cells lymphocyte proliferation	improved improved improved
McKay, 2000 [13]	free living healthy	39+41	67	28	multivitamin placebo	complete	8 weeks	plasma levels antioxidants cytokine production	improved = =
Mann, 1987 [14]	free living	48+53	64	-	multivitamin A, D, E, C, B1, B2, B6, niacin, B12, folate, panthotheenzuur, biotine, Fe, Cu, Mn, Zn placebo	5000 IU, 400 IU, 30 IU, 300 mg, 10 mg, 12 mg, 20 mg, 100 mg, 10 µg, 400 µg, 20 mg, 50 µg, 18 mg, 4 mg, 5 mg, 30 mg.	4 months	plasma levels	improved
Chandra, 1992,2001 [15,16]	free living apparently healthy	48+48	74	-	A, beta carotene, B1, B2, niacin, B6, folate, B12, C, D, E, Fe, Zn, Cu, Se, I, Ca, Mg placebo: Ca, Mg	400 RE, 16 mg, 2.2 mg, 1.5 mg, 16 mg, 3 mg, 400 µg, 4 µg, 80 mg, 4 µg, 44 mg, 16 mg, 14 mg, 1.4 mg, 20 µg, 0.2 mg, 200 mg, 100 mg	12 months	plasma levels T-cell subsets lymphocyte proliferation influenza vaccine morbidity memory	improved improved improved improved improved improved
De Jong, 2000,2001,2002[17-23]	free living frail	37+41	79	24	D, E, B1, B2, B6, folate, B12, C, Ca, Mg, Zn, Fe, I plus energy placebo: only energy	RDA	17 weeks	intake plasma levels anthropometry cognition function immunology bone density	improved improved = = = = improved
SINGLE NUTRIENT SUPPLEMENT									
Peretz, 1991 [24]	nursing home	11+11	78	26	selenium placebo	100 µg	6 months	plasma levels lymphocyte proliferation	improved improved
Meyer, 2002 [25]	nursing home frail	569+ 575	85	22	cod liver + vitamin D cod liver	10 µg	2 years	plasma levels fracture	improved =
Deijen, 1992 [26]	free living apparently healthy	38+38	73	-	vitamin B6 placebo	20 mg	12 weeks	plasma levels mood memory	improved = improved
Bogden, 1990 [27]	free living apparently healthy	24+20+ 19	71	27	zinc + multivitamin placebo: multivitamin	0, 15, 100 mg	12 months	dietary intake NK cells DTH lymphocyte proliferation	- improved reduced =

Author	Target group	N	Age (y)	BMI / *kg*	Intervention	Nutrients	Duration	Parameters	Outcome
Harman, 1986 [28]	institutionalized	52+26+25	-	-	vitamin E placebo	0, 100, 400 mg	6 months	influenza vaccination incidence infections	= =
Meydani, 1990[29]	free living healthy	14+18	65,70	-	vitamin E placebo	800 mg	30 days	plasma levels DTH lymphocyte proliferation	improved improved improved
Pallast, 1999 [30]	free living healthy	50+54+53	71	27	vitamin E placebo	0, 50, 100 mg	6 months	plasma levels DTH IL production	improved improved =
Waart, 1997 [31]	free living healthy	36+38	74	25-26	vitamin E placebo	100 mg	3 months	plasma levels lymphocyte proliferation	= =
Santos, 1997 [32]									
Studie I	free living healthy	12+11	70	-	beta carotene placebo	90 mg	23 days	DTH lymphocyte proliferation T-cell subsets	= = =
Studie II	free living healthy	27+27	63	-	beta carotene placebo	25 mg	10 years	DTH lymphocyte proliferation T-cell subsets	= = =
Smidt, 1991 [33]	free living no assistance	40+40	74	*60*	thiamin placebo	10 mg	6 weeks	dietary intake body weight plasma levels activity subjective assessment	improved improved improved = improved
Rydlewicz, 2002 [34]	free living	55+55+60+66+70+62	70	27	folic acid placebo	0,50,100,200,400, 600 µg	6 weeks	plasma levels Hcys	improved

Table 2b. *Overview of randomised, controlled intervention studies performed in elderly people.*

Author	Target group	N	Age (y)	BMI / *kg*	Intervention	Nutrients	Duration	Parameters	Outcome
Woo, 1994 [35]	hospital chest infection patients	40+41	73	20	liquid supplement control	500 ml 500 kcal	1 month	intake plasma levels body weight well-being	improved improved - improved
Potter, 2001 [36]	hospital emergency admissions	186+ 195	83	*49*	liquid supplement control	3*120 ml, 540 kcal	2 weeks	intake weight mortality ADL	improved improved improved improved
Joosten, 2001 [37]	hospital acute geriatric	50	83	25	liquid supplement control	300 kcal, 200 ml	13 days	intake	improved
Bos, 2001 [38]	hospital malnourished	6+17	78	21	liquid supplement control	2* 400 kcal	10 days	dietary intake plasma levels anthropometry grip strength	improved = improved =
Larsson, 1990 [39]	hospital	202+ 241	80	-	liquid supplement control	2*400 kcal	26 weeks	plasma levels weight anthropometry DTH mortality	improved improved improved improved improved
Lesourd, 1990 [40]	long stay hospital	24+24	81	19	liquid supplement control	400 kcal	3 months	plasma levels body weight anthropometry lymphocyte proliferation influenza vaccination	improved = = improved improved
Lauque, 2000 [41]	nursing home patients	22+13	85	22	liquid supplement control	300-500 kcal	2 months	energy intake body weight hand grip strength	improved improved =
Wouters-Wesseling, submitted	nursing home patients psychogeriatric	16+18	83	25	liquid supplement control	300 kcal	1 month	body weight anthropometry	improved =
Faxen-Irving, 2002 [42]	living group dementia	22+14	84	23	liquid supplements control	2*200 ml (410 kcal)	5 months	plasma levels body weight anthropometry MMSE + CDR ADL	= improved improved = =
Gray-Donald, 1995 [43]	free living need for home care	24+24	77	19	liquid supplement control	1000 kJ	12 weeks	energy intake body weight anthropometry handgrip strength well being	= + = = =

Author	Target group	N	Age (y)	BMI / *kg*	Intervention	Nutrients	Duration	Parameters	Outcome
Bunout, 2001 [44]	free living no chronic diseases	25+26	74	27	liquid supplement control	2*873 kJ	18 months	ADL body weight bone density	improved = improved
Chandra, 1985 [45]	free living apparently healthy	15+15	-	*59*	dietary advice + oral dietary supplements control	-	4 weeks	influenza vaccination	Improved
Krondl, 1999 [46]	free living healthy	36+35	70	24-25	liquid supplement control	235 kcal	16 weeks	dietary intake weight SF-36 well being	improved = improved improved

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Wendeline Wesseling was born in Ede, the Netherlands on May 27, 1970. In 1988 she graduated from secondary school at the Christelijk Streeklyceum in Ede. Thereafter she travelled in Europe for one year.

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