Nutrient-dense foods and exercise in frail elderly: effects on B vitamins, homocysteine, methylmalonic acid, and neuropsychological functioning[1–3]

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ABSTRACT

Background: Frail elders are at risk of suboptimal micronutrient status, functional decline, and neurologic disorders. The influence of oral multimicronutrients in physiologic doses and of moderately intense physical exercise on homocysteine (Hcy), methylmalonic acid (MMA), and neurologic functioning have not yet been investigated.

Objective: Our goal was to determine the effects of enriched foods and exercise on blood vitamins, Hcy, MMA, and neuropsychological functioning in the frail.

Design: A 17-wk randomized controlled intervention trial was used to study *1*) enriched foods plus a social program, *2*) regular foods plus exercise, *3*) enriched foods plus exercise, and *4*) regular foods plus a social program. Enriched foods contained multiple micronutrients (25–100% of the Dutch recommended dietary allowances); exercises focused on strength, coordination, flexibility, and endurance. Vitamin (cobalamin, red blood cell folate, and pyridoxal 5′-phosphate), Hcy, and MMA concentrations were measured and 2 neuropsychological tests were conducted.

Results: Vitamin concentrations were higher in the supplemented groups than in the unsupplemented groups ($P < 0.001$; total $n = 130$). Compared with baseline, cobalamin in the supplemented groups was increased by 22%, plasma folate by 101%, red blood cell folate by 87%, and pyridoxal 5′-phosphate by 68%. Concentrations in the unsupplemented groups changed by −2%, −6%, 1%, and −13%, respectively. Hcy decreased by 25% and MMA by 30% in the supplemented groups, compared with a small increase in Hcy (2%) and decrease in MMA (9%) in the unsupplemented groups. Exercise did not significantly affect vitamin, Hcy, or MMA concentrations. No significant effect of either intervention was observed on the neuropsychological tests.

Conclusions: The decrease in Hcy and MMA in frail elders confirms a subclinical metabolic deficiency state. Enriched foods containing physiologic amounts of micronutrients have a beneficial effect on these metabolites. No effects of B vitamins on mental health were identified. *Am J Clin Nutr* 2001;73:338–46.

KEY WORDS Frailty, elderly, intervention, enriched foods, exercise, homocysteine, methylmalonic acid, folate, vitamin B-12, reaction time, micronutrients

See corresponding editorial on page 151.

INTRODUCTION

Controversy exists as to whether low plasma concentrations of cobalamin (vitamin B-12), folate, and pyridoxal 5′-phosphate (PLP, or vitamin B-6) are prevalent with advancing age. Low concentrations of these vitamins in elderly subjects were reported by several but not all investigators (1–7). Other clinical indexes, such as plasma concentrations of homocysteine (Hcy) and methylmalonic acid (MMA), seem to be more functional indicators of intracellular vitamin deficiencies (7, 8). These indicators suggest that the prevalence of vitamin deficiency is substantially higher than predicted by plasma cobalamin, folate, and PLP concentrations (7, 9) and even provide metabolic evidence of vitamin deficiency in the presence of normal plasma vitamin concentrations (10).

Interest in elevated plasma Hcy and MMA concentrations as potential risk factors for cardiovascular disease, hematologic disorders, and neurologic disorders has grown notably. Deficiencies of folate, cobalamin, or both, and to a lesser extent of vitamin B-6, all of which are cofactors in Hcy- and MMA-metabolizing enzymes, may cause elevated concentrations of Hcy and MMA (2, 4, 11–13).

Frail elderly are particularly at risk of suboptimal or deficient micronutrient states, functional decline, and neurologic disorders. Primary or secondary factors influencing nutritional and health status are aging, chronic diseases (eg, atrophic gastritis), and physical inactivity, resulting in less energy expenditure and inadequate dietary intake or uptake (14–18). In the past decade, research in the frail elderly has shown the benefits of nutritional supplementation programs with and without exercise on some (but not all) indicators of nutritional and health status (19–22).

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TABLE 1
Dutch recommended dietary allowances (RDAs) and amounts of micronutrients in the enriched food products as a percentage of the Dutch RDA[1]

Micronutrient	RDA for men	RDA for women	Enriched food: Dairy product	Enriched food: Fruit product
			% of RDA[2]	
Vitamin D	7.5 μg (300 IU)	7.5 μg (300 IU)	100	—
Vitamin E	9.4 mg	8.3 mg	100	—
Thiamine	1.0 mg	1.0 mg	50	50
Riboflavin	1.5 mg	1.3 mg	50	50
Vitamin B-6	1.1 mg	1.0 mg	50	50
Folic acid	0.25 mg	0.25 mg	50	50
Vitamin B-12	2.5 μg	2.5 μg	50	50
Vitamin C	70 mg	70 mg	50	50
Calcium	0.9 g	0.9 g	25	—
Iron	9.0 mg	8.0 mg	25	25
Magnesium	0.33 g	0.28 g	12.5	12.5
Zinc	10.0 mg	9.0 mg	25	25
Iodine	0.23 mg	0.28 mg	50	50

[1] Dutch RDAs are from references 24 and 25.
[2] Per 100 g.

Nevertheless, the influence of orally administered multimicronutrients given in physiologic doses on Hcy and MMA concentrations has not been described in frail elderly. Likewise, the influence of exercise aimed both at a direct improvement in physical and neurologic functioning and at improvement in physical activity, energy expenditure, and, hence, total dietary intake, has not been taken into account.

For ethical reasons, proposed public health programs for lowering Hcy and MMA concentrations should not be based on unnecessarily high doses of B vitamins (23). To overcome any potential risk of overexposure with adverse effects, research is needed to determine the optimal dosages for community-based (frail) elderly who may have only mildly elevated metabolite concentrations. In this intervention trial, we focused on the effects of nutrient-dense foods containing physiologic doses of multiple micronutrients and of exercise on vitamin status, Hcy concentrations, MMA concentrations, and neuropsychological functioning in the frail elderly.

SUBJECTS AND METHODS

Subjects

The study population comprised 217 free-living Dutch frail elderly. The following criteria were used to select the study subjects: requirement of health care (such as home care or meals-on-wheels service), age ≥70 y, no regular exercise, body mass index (BMI; in kg/m^2) below average (≤25, based on self-reported weight and height) or recent weight loss, ability to understand the study procedures, and no use of multivitamin supplements. All subjects gave written, informed consent. The study protocol was approved by the external Medical Ethical Committee of the Division of Human Nutrition and Epidemiology of the Wageningen University.

Pre- and postintervention measurements were available for 165 subjects. Reasons for dropping out (n = 52, or 24%) were mainly health problems, including (terminal) disease, hospital admittance, and a recent fall or fracture. Valid (pre and post) vitamin measurements were available for 130 subjects. Four subjects were excluded because the time between the pre- and postintervention measurements was <13 wk as a result of hospitalization and 3 subjects could not visit our research center after the intervention because of illness. In one subject venipuncture was not successful. Additionally, 12 subjects were excluded from the analyses because of multivitamin use and another 14 were excluded because of vitamin B–complex use. Finally, one subject in the control group was dropped because of extremely high Hcy values (preintervention: 93.2 μmol/L, postintervention: 84.3 μmol/L).

Design

Enrollment took place between January and June 1997. Subjects were randomly assigned to 1 of 4 intervention groups: *1*) nutrition, consisting of nutrient-dense products plus a social program; *2*) exercise, consisting of regular products plus an exercise program; *3*) combination, consisting of nutrient-dense products plus an exercise program; and *4*) control (or placebo), consisting of regular products plus a social program. The intervention period was 17 wk. Data were collected at baseline (week 0) and after 17 wk (in week 18).

Nutrient-dense products

In addition to their usual diet, subjects were asked to consume 2 products daily: 1 from a selection of fruit products and 1 from a selection of dairy products. A variety of products were made available to the subjects to prevent boredom and to increase the acceptability of the products. Because of the limited shelf lives of the products, each participant received a new cooled container weekly with the following fresh servings of fruit-based products: 4 portions of orange-peach juice (portion size: 100 g), 4 portions of apple-berry-grape juice (100 g), 2 portions of apple compote (100 g), and 2 portions of apple-peach compote (100 g) and the following fresh servings of dairy products: 4 portions of vanilla custard (100 g), 4 portions of strawberry yogurt (100 g), 4 portions of vanilla-apple yogurt (100 g), and 4 portions of vanilla cheese curd with fruit (only 75 g because of the satiating effect of cheese curd).

Daily consumption of 2 enriched products delivered ≈100% of the Dutch recommended dietary allowance (RDA; 24, 25) of the following vitamins: D, E, thiamine, riboflavin, B-6, folic acid, B-12, and C and ≈25–100% of the Dutch RDA of the following minerals: calcium, magnesium, zinc, iron, and iodine (**Table 1**). Subjects in the control and exercise groups received identical regular products containing the usual amount of vitamins and minerals (≤15% of the concentrations in the enriched products) that could be purchased in supermarkets. Both the enriched and the regular products contained ≈0.48 MJ energy per 2 products.

Exercise program

The main objective of the exercise program was maintenance of or improvement in mobility and performance of daily activities essential for independent functioning through maintenance of versatility in movement. Emphasis was placed on skills training; muscle strength, coordination, flexibility, speed, and endurance were developed through exercises such as walking, stooping, and chair stands. Different materials were used, eg, balls, ropes, weights, and elastic bands. Group sessions were organized twice weekly, lasted 45 min, and were of moderate,

gradually increasing intensity. The sessions were coordinated by skilled teachers and supervised by one of the project leaders (MCAP). To guarantee uniformity, all sessions were rehearsed extensively with all teachers together; moreover, an instruction video and manual were made in advance.

A social program served as a control (for attention) program for the exercise program. Sessions lasting 90 min were organized once every 2 wk by a skilled creative therapist. This program focused on creative activities, social activities, and lectures about topics of interest to the elderly. Transport to and from all sessions was arranged.

Measures

Questionnaires were used to collect information on age, sex, marital status, education, living conditions, illness, medicine and supplement use, and physical activity level. Body weight was measured to the nearest 0.05 kg on a digital scale (ED-6-T; Berkel, Rotterdam, Netherlands) and height was measured to the nearest 0.001 m with a wall-mounted stadiometer. Body mass index was calculated as weight in kilograms divided by height in meters squared (26).

Neuropsychological indexes

Two validated tests were used to measure neuropsychological functioning. In the first, a block-transfer test, each participant was instructed to replace 40 blocks with the preferred hand from a full board to an empty board as fast as possible in a prescribed sequence. In the second test, of reaction time, each subject had to react to the onset of a light by pushing a button as fast as possible. The reaction time test was performed 15 times and the median value of the 15 results was used in further analyses. In both tests, a psychomotor component was present [eg, transcription of information (the visual observation) by the central nervous system] in addition to the motor component (eg, replacing blocks and pushing a button). Details of these 2 tests can be found elsewhere (27).

Biochemical indexes

Blood samples from fasting subjects were collected between 0700 and 0900 for measurement of all indexes except the complete blood count and Hcy concentration. For practical reasons, samples used to measure Hcy and the complete blood count were collected in our research center at 1200 and put on ice immediately before further processing. For the measurement of total Hcy, 0.5 mL nonfasting, EDTA-treated blood was used for analysis (HPLC-fluorometry). All samples were analyzed within one run, with a CV of 3.5%. A fresh 3 mL of nonfasting, EDTA-treated blood was used for measurement of the complete blood count (Coulter Counter type T-860; Coulter Electronics, Miami). MMA was measured in 500 μL serum by use of gas chromatography–mass spectrometry. The between-assay CV was 9%.

Of the fasting blood samples, 1.5 mL EDTA-treated blood was preserved for analyses of vitamin B-12 and folate by ion-capture IMx (Abott Laboratories, Abott Park, IL) (28, 29). Between-run CVs were <5% and <10%, respectively. For measurement of red blood cell folate, hemolysates were prepared from 100 μL fasting, EDTA-treated plasma and 2 mL 1% ascorbic acid in water. Lysis reagent containing guanidine HCl was used to dilute the sample 4 times and was added at the time of analysis. Ion-capture IMx was followed by calculation of corrected red blood cell folate concentration with use of the hematocrit and plasma folate concentration in the formula. A regression formula was used to correct for the delayed dilution with lysis reagent. CVs for red blood cell folate were <13% between runs. For vitamin B-6, PLP was measured (derivatives were separated by reversed-phase HPLC and detected by fluorescence) in 1.0 mL plasma (26). The between-assay CV was 5–10%.

All samples were stored at −80°C until analyzed. Pre- and postintervention samples were analyzed in the same batch. Analyses were performed by the TNO Nutrition and Food Research Institute, Zeist, Netherlands (PLP); the Department of Clinical Chemistry, University Hospital Nijmegen, St Radboud, Nijmegen, Netherlands (vitamin B-12 and plasma and red blood cell folate); University Hospital, Free University, Amsterdam (MMA); and the division of Human Nutrition and Epidemiology, Wageningen University, Wageningen, Netherlands (hemocytometry and Hcy).

Statistical analysis

Data were analyzed by using the statistical program SAS (version 6; SAS Institute Inc, Cary, NC). Means ± SDs, medians (and 10th to 90th percentiles), or percentages of (baseline) values were calculated for all intervention groups. Log-transformed data were used for MMA because the distribution of this variable was markedly skewed. Means and mean changes (±SDs) per intervention group were calculated and compared with mean changes in the control group by analysis of variance followed by Tukey's procedure. Dropouts were compared with participating subjects by unpaired *t* tests. The prevalences of subjects deviating from reference deficiency values before and after the intervention were calculated as frequencies, ie, vitamin B-12 < 221 pmol/L, PLP < 20 nmol/L, plasma folate < 6.3 nmol/L, red blood cell folate < 337 nmol/L, MMA > 0.35 μmol/L, and Hcy > 16.2 μmol/L according to the manufacturers' stated lower limits of normal values and published sources (4, 9, 10, and 30–33). With respect to Hcy, data and cutoffs for fasting and nonfasting subjects were described in the literature (4, 9, 30); with respect to MMA, concentrations between 0.32 and 0.38 μmol/L were reported (4, 9, 30). For the 2 neuropsychological tests, medians and median changes (with 10th to 90th percentiles) per intervention group were calculated and compared with the control group (rank-sum test).

Spearman partial correlation coefficients were obtained between (baseline) blood vitamin values, Hcy, and MMA concentrations and the neuropsychological tests, adjusted for age effects. Multiple regression was used to determine the effect of both interventions and a possible interaction on the change in Hcy and MMA. Because no evidence of interaction was observed, a comparison was made between the supplemented groups (the nutrition and combination groups) and the unsupplemented groups (the exercise and control groups) and between the exercise groups (the exercise and combination groups) and the nonexercise groups (the nutrition and control groups) by linear regression. Baseline values for Hcy, MMA, B vitamins, and age were added as covariates to the model to check for confounding.

RESULTS

The baseline characteristics of each intervention group are presented in **Table 2**. Seventy-one percent of the population was female. The subjects' mean age was 78 y, their mean BMI was 24, and their mean subjective health score was 6.9 as rated on a 10-point scale (1 being very unhealthy and 10 being very

TABLE 2
Baseline characteristics of the frail elderly study population according to intervention group[1]

Characteristic	Control group (n = 30)	Exercise group (n = 31)	Combination group (n = 33)	Nutrition group (n = 36)
Women (%)	67	74	76	69
Age (y)	79.0 ± 7.2[2]	76.9 ± 4.6	77.9 ± 5.2	78.8 ± 4.8
Weight (kg)	66.9 ± 10.9	65.2 ± 12.1	67.6 ± 8.0	66.1 ± 8.9
BMI (kg/m^2)	24.5 ± 3.2	24.1 ± 3.2	24.8 ± 2.7	24.3 ± 2.4
Subjects living alone (%)	67	71	73	67
Activity score[3]	59 (34–110)[4]	55 (27–89)	62 (30–115)	61 (34–103)
Subjective health score[5]	7.0 ± 1.5	6.9 ± 1.3	6.9 ± 1.3	6.9 ± 1.7
No. of prescribed medicines	2 (0–5)	2 (0–6)	2 (0–5)	3 (0–6)
Subjects reporting ≥1 disease (%)	83	94	94	86

[1] There were no significant differences between groups.
[2] $\bar{x}$ ± SD.
[3] Physical Activity Scale for the Elderly (34); range: 0–400.
[4] Median (10th percentile to 90th percentile).
[5] Range: 1–10.

healthy). No significant differences among the 4 intervention groups were observed.

The study dropouts did not differ significantly from the subjects who successfully completed the trial. The dropouts' mean age (n = 43) was 79.5 ± 5.2 y, their mean subjective health score (n = 43) was 6.5 ± 1.5, their mean BMI (n = 37) was 24.2 ± 2.4, and their mean baseline Hcy concentration (n = 38) was 16.9 ± 6.2 μmol/L. The dropouts' (n = 45) median score on the block transfer test was 56 s and their median score on the reaction time test was 242 ms.

The mean dietary intake of selected vitamins and energy and the changes in intake observed after 17 wk are presented in **Table 3**. In general, intakes in both the nutrition and the combination group increased significantly compared with intakes in the control group.

Mean concentrations of vitamins, Hcy, 3 hematologic indicators, and MMA and the changes observed after 17 wk are shown in **Table 4**. Baseline values were not significantly different among the 4 groups. On average, one-half of the study population had baseline concentrations of Hcy above the reference value of 16.2 mmol/L. About 40% of the participants had MMA concentrations >0.35 μmol/L. Beneficial changes in concentrations of Hcy, MMA, and B vitamins were reflected in shifts in the percentages of participants above or below the reference values as well as in alterations in mean or median concentrations in both the combination and the nutrition groups compared with the control group. No significant differences in change in concentrations of vitamins, Hcy, or MMA were observed between the exercise group and the control group.

With respect to the hematologic data at baseline, values for only a few subjects were outside reference values [ie, hemoglobin < 120 g/L (women) or 130 g/L (men) and hematocrit < 0.36 (women) or 0.40 (men)]. There were no significant changes in hematologic data in any of the intervention groups after 17 wk.

To investigate whether any subjects persistently did not respond to nutritional intervention, we examined the individual results of those subjects with high postintervention Hcy and MMA concentrations. Within the supplemented groups (the nutrition and combination groups), we found 11 participants with a postintervention Hcy concentration above the reference value. In 2 of these subjects, Hcy concentrations had increased despite the intervention. Most of these subjects showed a decline in MMA, an increase in vitamin B-12, or both [MMA was not

TABLE 3
Mean dietary intakes of energy and relevant vitamins and 17-wk changes in the frail elderly study population[1]

Nutrient	Control group (n = 30)	Exercise group (n = 30)	Combination group (n = 32)	Nutrition group (n = 36)
Energy (MJ/d)				
Baseline	7.8 ± 2.2	6.9 ± 1.4	7.1 ± 1.5	7.8 ± 1.8
Change	−0.3 ± 2.0	0.3 ± 1.1	−0.04 ± 1.2	−0.3 ± 1.4
Vitamin B-12 (μg/d)				
Baseline	3.5 ± 2.1	2.9 ± 1.2	3.4 ± 1.3	3.5 ± 1.5
Change	0.7 ± 2.5	1.0 ± 3.2	2.1 ± 1.6	3.6 ± 4.2[2]
Folic acid (mg/d)				
Baseline	0.26 ± 0.09	0.23 ± 0.06	0.22 ± 0.05	0.24 ± 0.09
Change	−0.02 ± 0.07	−0.10 ± 0.07	0.26 ± 0.09[2]	0.29 ± 0.13[2]
Vitamin B-6 (mg/d)				
Baseline	1.3 ± 0.3	1.6 ± 2.1	1.2 ± 0.3	1.3 ± 0.4
Change	−0.01 ± 0.4	−0.3 ± 2.2	1.2 ± 0.4[2]	1.2 ± 0.7[2]

[1] $\bar{x}$ ± SD.
[2] Significantly different from the control group, $P < 0.001$.

TABLE 4
Mean blood vitamin concentrations and 17-wk changes in the frail elderly study population[1]

Variable	Control group (n = 30)	Exercise group (n = 31)	Combination group (n = 33)	Nutrition group (n = 36)
Vitamin B-12 (pmol/L)				
Baseline	229 ± 87.2[2]	233 ± 97	299 ± 142	281 ± 113[3]
Change	−2 ± 37	−5 ± 42	51 ± 72[4]	78 ± 67[4]
Percentage <221 pmol/L (%)				
Preintervention	50	55	30	42
Postintervention	47	52	18	11
Folate (nmol/L)				
Baseline	16.4 ± 7.0	19.9 ± 8.1	18.0 ± 7.2[5]	19.0 ± 7.8
Change	−0.15 ± 3.2	−2.1 ± 4.9	19.5 ± 6.8[4]	18.0 ± 7.2[4]
Percentage <6.3 nmol/L (%)				
Preintervention	0	0	0	0
Postintervention	0	0	0	0
Red blood cell folate (nmol/L)				
Baseline	540.5 ± 134.4	462.9 ± 146.9	491.6 ± 180.2[5]	502.1 ± 227.6
Change	−10.8 ± 190.1	22.8 ± 121.2	376.8 ± 177.8[4]	488.0 ± 293.8[4]
Percentage <337 nmol/L (%)				
Preintervention	10	23	22	28
Postintervention	17	13	0	0
PLP (nmol/L)				
Baseline	32.3 ± 22.4	33.8 ± 22.7	35.8 ± 31.7	38.3 ± 25.1
Change	−3.9 ± 13.5	−0.5 ± 28.8	17.7 ± 27.0[4]	32.6 ± 29.4[4]
Percentage <20 nmol/L (%)				
Preintervention	23	26	30	14
Postintervention	23	19	3	0
Homocysteine (μmol/L)				
Baseline	19.5 ± 7.8	16.8 ± 7.0[6]	16.3 ± 6.1	17.3 ± 5.7
Change	0.3 ± 5.6	0.5 ± 2.4	−4.6 ± 4.8[4]	−4.0 ± 3.9[4]
Percentage >16.2 μmol/L (%)				
Preintervention	57	45	48	53
Postintervention	63	45	9	22
MMA (μmol/L)				
Baseline	0.39 (0.19–0.65)[7]	0.28 (0.16–0.58)[6]	0.27 (0.13–0.55)[5]	0.32 (0.17–0.70)[3]
Change	−0.02 (−0.17–0.25)	−0.04 (−0.26–0.22)	−0.06 (−0.30–0.10)	−0.11 (−0.36–0.04)[8]
Percentage >0.35 μmol/L (%)				
Preintervention	57	32	33	36
Postintervention	50	23	15	14
Hemoglobin (mmol/L)				
Baseline	8.5 ± 0.7	8.7 ± 0.7	8.9 ± 0.7	8.6 ± 0.7
Change	−0.2 ± 0.4	−0.3 ± 0.3	−0.3 ± 0.3	−0.2 ± 0.3
Hematocrit				
Baseline	0.41 ± 0.03	0.42 ± 0.03	0.43 ± 0.03	0.42 ± 0.03
Change	−0.01 ± 0.02	−0.00 ± 0.01	−0.01 ± 0.01	−0.01 ± 0.02
Mean corpuscular volume (fL)				
Baseline	92 ± 4	91 ± 4	91 ± 4	92 ± 4
Change	0.3 ± 1.6	0.2 ± 1.1	1.0 ± 1.5	0.5 ± 1.7

[1] PLP, pyridoxal 5′-phosphate; MMA, methylmalonic acid.
[2] $\bar{x}$ ± SD.
[3] n = 35.
[4,8] Significantly different from control group: [4] $P < 0.001$, [8] $P < 0.05$.
[5] n = 32.
[6] n = 30.
[7] Geometric mean (10th to 90th percentile).

decreased in 4 subjects (it remained the same in 1); however, of these subjects, 3 had increased vitamin B-12 concentrations]. Only 1 of the 11 subjects had a postintervention vitamin B-12 concentration below the reference value, although this subject's vitamin B-12 concentration had increased by 10 pmol/L. Likewise, we found 10 subjects with an MMA concentration >0.35 μmol/L after the intervention. Hcy concentrations had decreased in all 10 of these subjects (by 5.4 ± 4.5 μmol/L). Four subjects had increased MMA concentrations despite the intervention; of these, 3 had an improved vitamin B-12 concentration (with a mean change of 43 pmol/L). Only 4 persons overlapped with the group who still had high Hcy concentrations.

We found no interaction between the exercise program and nutritional supplementation; therefore, effects were analyzed separately. The difference in change in Hcy concentrations between the supplemented groups and the unsupplemented groups was

TABLE 5
Difference in change in homocysteine and methylmalonic acid concentrations in the frail elderly study population, according to type of intervention

Variable	Nutrient-dense foods compared with regular foods: difference (95% CI)	Exercise compared with no exercise: difference (95% CI)
Homocysteine, n = 129 (μmol/L)		
Crude	−4.4 (−5.8, −3.1)	−0.04 (−1.4, 1.3)
Adjusted[1]	−5.0 (−6.0, −3.9)	−0.7 (−1.8, 0.4)
Methylmalonic acid, n = 127 (μmol/L)		
Crude	−0.09 (−0.17, −0.01)	0.02 (−0.06, 0.10)
Adjusted[2]	−0.10 (−0.17, −0.03)	0.01 (−0.06, 0.08)

[1] Adjusted for baseline homocysteine and folate concentrations.
[2] Adjusted for baseline methylmalonic acid concentration and age.

5.0 μmol/L in the adjusted model. For MMA, the difference was 0.1 μmol/L in the adjusted model (**Table 5**). We found no significant benefit of exercise on Hcy or MMA concentrations.

Baseline median scores and changes in scores on the 2 neuropsychological tests are presented in **Table 6**. No significant differences between groups were found at baseline, nor were any significant differences in change compared with the control group detected. When the exercise groups were compared with the nonexercise groups in the multiple regression analysis, the differences in changes remained insignificant (data not shown).

Baseline Hcy concentration was inversely correlated (when adjusted for age) with vitamin B-12 ($r = -0.41$, $P < 0.0001$) and plasma folate ($r = -0.54$, $P < 0.0001$), inversely correlated (although only borderline) with red blood cell folate ($r = -017$, $P = 0.06$), and not correlated with PLP ($r = -0.13$, $P = 0.15$). Baseline MMA was correlated with Hcy ($r = 0.39$, $P = 0.0001$) and inversely correlated with vitamin B-12 ($r = -0.39$, $P < 0.0001$). No significant correlations were found between baseline MMA and red blood cell folate or vitamin B-6. Furthermore, only Hcy was related to scores on the neuropsychological tests (both tests: $r = 0.20$, $P = 0.03$). The 17-wk change in Hcy was inversely correlated with change in folate (plasma folate: $r = -0.67$, $P < 0.001$; red blood cell folate: $r = -0.54$, $P < 0.001$). Changes in vitamin B-12 and PLP also were inversely correlated with Hcy, but to a lesser extent ($r = -0.40$, $P < 0.0001$ and $r = -0.42$, $P < 0.001$, respectively). Significant correlations were found between change in MMA and change in vitamin B-12 ($r = -0.22$, $P = 0.01$), plasma folate ($r = -0.20$, $P = 0.03$), and red blood cell folate ($r = -0.27$, $P = 0.003$), but not with PLP ($r = -0.08$, $P = 0.37$). Changes in concentrations of vitamins, Hcy, or MMA were not significantly correlated with changes in scores on the neuropsychological tests (data not shown).

DISCUSSION

The 17-wk consumption of foods containing physiologic amounts of micronutrients beneficially affected blood vitamin, Hcy, and MMA concentrations in Dutch frail elderly. Considerable decreases in Hcy of 25% and in MMA of 21% in the supplemented groups compared with the unsupplemented groups confirm a subclinical metabolic deficiency state in the frail elderly we studied. There was no significant effect of the enriched foods on the results of 2 tests indicative of neuropsychological functioning. Also, no significant effect was observed on any of the measured indexes of all-round moderately intense exercise, nor was there an interaction between the enriched foods and exercise.

Moderately increased Hcy and MMA concentrations and low B vitamin concentrations may cause cardiovascular disease, neuropsychiatric damage, or hematologic abnormalities (9, 35–37). Meta-analyses of observational studies predict that a 1-μmol/L decrease in Hcy can result in a 10% reduction in risk of coronary artery disease. This prognosis was based on Hcy concentrations within the range of 10–15 μmol/L (36). Baseline Hcy values in our population exceeded this range, and the question remains whether this prediction is valid for such an old population. Data are needed on the implication of a 10% reduction in old age. Another implication of increasing B vitamin status and lowering associated metabolites in an elderly population may be the effect of such changes on mental health. Until now, predictive data on the possible effect of interventions on neurologic functioning were lacking.

A comparison of our data with values measured in other free-living and hospitalized elderly populations shows that Hcy, vitamin B-12, and folate concentrations were higher in our group (10, 11, 30, 38–40). Similar vitamin B-12 values were found in geriatric patients with folate and vitamin B-12 deficiencies (41). Our MMA data are in line with data for Dutch elders classified between possibly and mildly cobalamin deficient (4) but are higher than values measured in hospitalized patients (10). Yet, compared with measurements in other studies, our values were less unfavorable or at least similar (2, 10, 11, 40–42). This illustrates the striking variability in observed vitamin concentrations and interpretation of reported concentrations as indicative of deficiencies. A partial explanation is the use of different methods and cutoffs. For evaluation of folate deficiency, predominantly method-specific reference ranges have been indicated (43). Only recently was raising the screening concentration for B-12 deficiency suggested (4, 30, 31).

TABLE 6
Median outcomes of neuropsychological tests and 17-wk median change in the frail elderly study population[1]

Test	Control group (n = 30)	Exercise group (n = 31)	Combination group (n = 33)	Nutrition group (n = 36)
Reaction time (ms)				
Baseline	255 (206–338)	250 (201–309)	222 (183–361)	229 (177–403)
Change	−15 (−77 to 51)	−2 (−44 to 51)	−2 (−71 to 69)	8 (−84 to 65)
Block transfer (s)				
Baseline	56 (48–72)	55 (48–67)	54 (43–68)	57 (49–73)
Change	−0.8 (−11 to 8)	−2 (−8 to 3)	−2 (−8 to 7)	0.1 (−8 to 9)

[1] Median (10th percentile to 90th percentile). A negative change indicates improvement.

A few reports mentioned metabolic evidence of vitamin deficiency in the elderly in the presence of normal plasma vitamin concentrations (7, 10); investigators have now reached a consensus that it is more appropriate to focus on functional indexes than solely on blood vitamin concentrations (10, 44). In our population, relatively high Hcy concentrations were accompanied by deficiencies in plasma vitamin B-12 but not folate in a considerable number of subjects. This higher prevalence of vitamin B-12 deficiency than of folate deficiency agrees with the findings of Hanger et al (2) and the results of the Framingham study (9) but not with the findings of Ortega et al (11, 45). The overall decline in total Hcy and MMA in the supplemented compared with the unsupplemented groups confirms once more the need for examining metabolic deficiency even in the absence of low vitamin concentrations. Perhaps the current cutoffs for folate deficiency should be reevaluated.

Significant beneficial changes in hematologic indexes were not detected. Despite depletion of folate or vitamin B-12, anemia induced by this depletion occurs only at the far end of the vitamin deficiency spectrum. As a consequence, it is extremely difficult to prove beneficial effects of supplementation on hematologic indexes in subjects who are only mildly deficient (46).

Elderly persons who are deficient in vitamin B-12 may develop a wide variety of neurologic abnormalities (44, 47) but, until now, large-scale, well-controlled studies in the elderly did not show overall improvements in neuropsychological function after therapy (1). We also did not find a baseline relation between our tests of neuropsychological functioning and vitamin B-12 status, nor did we find improvement in test scores with supplementation. The reliability and validity of the 2 tests were judged earlier to be sufficient on a group level, according to criteria of objectivity, stability, consistency, and relative validity with respect to other tests. In a random sample of independently living elderly (aged $\geq$50 y), test-retest and concurrent validity correlation coefficients varied between 0.83 and 0.92 and between 0.46 and 0.75, respectively (27, 48). All of our participants could perform the tests, which is at least a strong indicator of the appropriateness of these tests in a frail population. Although our baseline values were below the performance of moderately active and sedentary Dutch healthy subjects (49), and despite the fact that we had a considerable proportion of B-12–deficient subjects and metabolic evidence that supplementation had a beneficial effect on MMA and Hcy, the lack of improvement in neuropsychological functioning may indicate that the deficiency was not severe enough or the degenerating process had not developed far enough. A degenerating process as such might not even be reversible. Also, 17 wk of consumption of enriched foods containing physiologic amounts of micronutrients may not have been long enough. Subtle neuropsychological improvements may perhaps be detected only with more sensitive tests or in more deficient patients.

A few studies showed a lowering of plasma Hcy and MMA by intramuscular administration of pharmacologic doses of folic acid, vitamin B-12, vitamin B-6, or all 3 (10, 23). The effect of lower doses in the elderly in particular has not been studied until now but is recognized as being highly relevant to the question of food fortification. Ward et al (23) reported an effective lowering of Hcy concentrations in apparently healthy volunteers given a daily dose of 200 μg but not 100 μg folic acid. The effectiveness of relatively low doses of folic acid was confirmed by others (50). Also, Garry et al (3) concluded that significant increases in folate and vitamin B-12 concentrations could be achieved through dietary supplements. This finding, however, was questioned by others who found only modest correlations between supplement intake and serum metabolite concentrations (9). With respect to frail elders with mildly elevated Hcy and MMA concentrations, we found a noteworthy favorable decline in these metabolites induced by micronutrient-enriched foods. Although we still observed a few subjects with adverse postintervention concentrations of vitamins or metabolites, this finding could not be attributed to a persistent nonresponse to treatment (ie, persistent malabsorption).

Low doses are more likely to be achieved through food enrichment, which is a more attractive way of increasing B vitamin status in the elderly than are tablets, injections, or increases in total food intake. Simultaneous administration of folic acid and vitamin B-12 is preferred over the administration of folic acid alone (9) because inappropriate mistreatment of clinical vitamin B-12 deficiency should be precluded.

In the present study, the exercise program did not have an additive effect on blood vitamins or any metabolite. Exercise was assumed to act through increased dietary intake. Changes in total energy intake indeed differed significantly between the exercise and nonexercise groups, but this was attributed mainly to a decline in intake in the latter group (51). Training of cognitive function, as measured by neuropsychological tests, could have caused a direct beneficial effect as well. The small improvement on these tests was observed in both the exercise and nonexercise groups and therefore was not a direct training effect.

The beneficial effects on Hcy and MMA of nutrient-dense foods containing physiologic amounts of micronutrients seem relevant, although optimal dosages still need to be defined. Enriched foods may be an easily adopted, attractive, and inexpensive intervention for improving nutritional status in frail elders. The beneficial decline in risk of cardiovascular disease and comorbidity and the relation with mental health in a population at this age should be investigated in the long term.

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