Effect of micronutrient pack on micronutrient status and antioxidant capacities among institutional older adults in Shanghai, China

Running title: Effect of micronutrient pack intervention

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ABSTRACT

Background and Objectives: Older adults are at increased risk of micronutrient deficiency, disrupting the balance of oxidation/antioxidation system and leading to serious health burdens. This study aimed to investigate the effect of micronutrient pack on micronutrient status and oxidative/antioxidative biomarkers in institutional older adults. **Methods and Study Design:** Subjects aged 65-100 years were randomly assigned to either intervention group or control group (n=49 each), providing a package of micronutrient pack or placebo daily for three months. The concentrations of micronutrients, malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) were detected both at baseline and at the end of the study. **Results:** The changes in concentrations of serum folate (21.1±1.6 vs 0.6±0.5 nmol/L), vitamin B-1 (3.4±0.4 vs -0.2±0.3 nmol/L), vitamin B-2 (11.5±3.3 vs 2.3±1.4 nmol/L), vitamin B-12 (128.8±34.8 vs 13.3±16.0 pmol/L), 25-hydroxyvitamin D (17.8±1.3 vs -0.8±0.5 ng/mL) and plasma zinc (0.6±1.8 vs -9.6±1.9 μmol/L) over 3-months were significantly increased in the intervention group compared with the control group (all $p<0.05$). While the prevalence of folate, vitamin B-12 and vitamin D deficiencies were significantly decreased after 3-months intervention (all $p<0.05$). Moreover, changes in serum MDA level (-1.5±0.2 vs 0.2±0.3 nmol/mL) were remarkably reduced, and the activities of serum GSH-Px (1.3±0.3 vs 0.3±0.2 ng/mL) and plasma SOD (14.3±2.4 vs -2.1±2.4 U/mL) were increased in the intervention group than those of in the control group (all $p<0.01$). **Conclusions:** The micronutrient pack among institutional older adults was well-accepted with good compliance and tolerance. The 3-month intervention may improve micronutrient status and enhance antioxidative capacities.

INTRODUCTION

Unlike the protein-energy malnutrition, micronutrient deficiency (MND) is less visible and usually begins to show when the health condition is severe, and is known as "hidden hunger".[1] Due to the age-related physical changes, older adults face more serious challenges in the attainment of appropriate micronutrients.[2] Previous studies have shown that insufficient dietary intake of vitamin C, vitamin D, vitamin B-6, vitamin B-12, folate, calcium, magnesium, zinc, and selenium,[3-5] and high prevalence of folate, vitamin B-12 and vitamin D deficiency[6-8] are very common in the older adults.

MND can lead to serious health issues in older adults, including alterations in immune response, declined cognitive function and physical performance, decrease in the quality of life, increase in the risk of chronic diseases, hospitalization, morbidity, and even mortality.[9-11] Moreover, several studies demonstrated that MND could disrupt the balance of oxidation and anti-oxidation system.[12-14] Hence, it is theoretically possible that MND may enhance the declination of geriatric function through exaggerating the oxidative stress.

As an approach to prevent MND, micronutrient powders (micronutrient pack for young children, Chinese Child Improvement Program) have been proved to be effective in improving the nutritional status of children aged 6-23 months.[15] Different from the pills or capsules for the correction of MND, this micronutrient pack is a food-based micronutrient mixture powder, and is provided in a single-served packet with the aim to complete the dietary intake of micronutrients according to dietary reference intakes (DRIs).[16] However, whether this approach can be applicable to older adults has not been investigated till date. In the former studies by our research group, the dietary intakes of calcium, iron, zinc, selenium, vitamin B-1, vitamin B-2 and vitamin C among the institutional older adults (n=600) were shown to be less than the Chinese DRIs.[5,17] To address this gap, a dietary supplement, micronutrient pack was designed to complement the insufficient dietary intakes of micronutrient in order to meet the requirements of DRIs.

Therefore, in the present study, a randomized, double-blind, placebo-controlled trial was performed to assess the effect of 3-month supplementation with micronutrient pack on blood micronutrient status and oxidative/antioxidative biomarkers among the older adults living in long-term care facilities (LTCFs).

MATERIALS AND METHODS

Study Design and Participants

This was a 3-month randomized, double-blind, placebo-controlled trial, which was conducted between February 2017 and November 2017 in three LTCFs (Yinyuan Hospital, Donghai Geriatric Nursing Hospital and Social Welfare Institute of Minhang District) in Shanghai, China. The study protocol was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of Shanghai Nutrition Society (No. 2017-001). Written informed consent was obtained from all participants. This trial was registered at the Chinese Clinical Trial Registry (http://www.chictr.org.cn) as ChiCTR-IOR-17010876.

Eligible participants included older adults aged between 65 and 100 years of both genders with poor nutritional status (PNS) according to the Mini Nutritional Assessment short-form

(MNA-SF). PNS was defined as malnutrition or being at risk of malnutrition with MNA-SF score≤11 points.[18] Subjects who used supplements or injected vitamins or minerals during the past six months; were bedridden or unconscious; previously underwent gastrointestinal surgery; had severe hearing loss; or currently suffering with acute infection were excluded.

Micronutrient pack and placebo

The micronutrient pack was designed to complement the insufficient dietary intakes of the institutional older adults to reach Chinese DRIs. Based on the previous investigation on the dietary intakes among 600 older adults in the above three LTCFs,[17] the composition was determined by discussing with experts from Chinese Nutrition Society (Table 1). The dietary intakes of vitamin B-6, vitamin B-12, folate and vitamin D could not be attained, as they are not included in the China's food composition tables. So, 2/3 DRIs of vitamin B-6, vitamin B-12, and folate, and tolerable upper intake levels of vitamin D were selected as the supplement dose. One package of micronutrient pack weighing 20 g with 244 kJ was supplied in powdered form with micronutrients. The placebo consisted of the same composition without micronutrients. Considering the taste preferences of the older adults, the formula was prepared based on soybean protein, whey protein, maltodextrin, and rice powder, giving a corn and milk mixed aroma with faint sweet taste. The micronutrient pack was canary yellow because of the addition of β-carotene, while the placebo was off-white. To ensure the blindness, the supplements were packed in an identical opaque packaging, and could be distinguished only by the group number printed on the surface. All the ingredients were soluble and so both the micronutrient pack as well as the placebo could be easily dissolved in water, juice, milk, or porridge. All supplements were manufactured by Richen Nutritional Co., Ltd (Shanghai, China).

Interventions

Eligible subjects were randomly assigned (1:1) to the intervention group (n=49) and control group (n=49) to receive either a packet of micronutrient pack or placebo once daily for three months. All the supplements were dispensed by the dieticians weekly. The participants were asked to add the supplements in milk, yogurt or porridge to eat and take during or immediately after a meal. To monitor the compliance, the amount of supplement intake was checked by counting the packages that has been taken at weekly intervals, and all unused supplements were returned to the counter at the end of the study. At each regular visit, the subjects were specifically asked if they had any discomfort during the intervention. The

randomization sequences were computer-generated by an independent statistician. All the investigators and participants in this study were blinded to the treatment assignments.

Data collection

Demographic data including age and educational status were collected at baseline by using a questionnaire. The body mass index (BMI) was calculated by weight and height and was measured by experienced research nurses. Three consecutive 24-hour dietary recalls combined with weighing method were used to assess the dietary intakes at baseline and the end of intervention (3-month).[19] The trained dietitians collected the detailed information of all foods and beverages consumed by the subjects in the past 24 h. Dietary intakes of nutrients were calculated using Nutri-star Data System (Shanghai Zhending Inc., Shanghai, China) software. All subjects were requested to keep their eating habits unchanged throughout the study.

Biochemical measurement

Fasting serum and plasma samples at baseline and 3-months (end line) were collected, centrifuged, separated and stored at -80°C before tested. All the samples were analyzed within one week of collection. The concentrations of serum iron and ferritin were tested at the clinical laboratory of Yinyuan Hospital with routine measurements. Other biochemical analyses were carried out at the Adicon clinical laboratory, Shanghai, China. The serum concentrations of vitamin A, vitamin C, vitamin B-1, vitamin B-2, vitamin B-6, malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) were determined using the commercial kits (Shanghai Lengton Bioscience Co., LTD, Shanghai, China) following the manufacturer's instructions. The plasma zinc and magnesium concentrations were analyzed by atomic absorption spectrometry.[20] Plasma superoxide dismutase (SOD) was analyzed by colorimetric method,[21] and serum 25-OHD-3 by mass-spectrography method,[22] respectively. Serum folate and vitamin B-12 were analyzed by electrochemiluminescence method.

Sample size

As a subclinical or mild folate deficiency is common in older adults,[23] the required sample size was calculated by assuming a substantial increase from 15.91±8.26 nmol/L (7.01±3.64 ng/mL) to 20.36±1.41 nmol/L (8.97±0.62 ng/mL) in the plasma folate after 6-months of oral supplementation with 400 μg of folate daily in Chinese older adults.[24] Based on the results of previous study, using a two-tailed alpha of 0.05 and power of 90%, it was determined that at

least 30 participants were required for each group to detect a significant change in folate by 4.45 nmol/L. With an estimated potential attrition rate of 20%, the required sample size was estimated to be greater than 38 participants in each group (76 participants in total).

Statistical analyses

All study data were analyzed using the SPSS software (version 24; IBM). Categorical variables were reported as numbers (n) and percent (%), and Chi-square tests were performed to analyze the differences between the two groups. Continuous data were reported by means and standard deviations, and the normality of distribution was tested using the Kolmogorov-Smirnov test. The primary outcomes were 3-month mean (95% CI) changes in blood micronutrient concentrations. Secondary outcomes were 3-month changes in blood MDA, SOD and GSH-Px between the two groups. Data from baseline to 3-month (3-month - baseline) were calculated within the intervention group and within the treatment group. The normally distributed variables were analyzed by independent-sample t-tests, and skewed distributed variables by Mann-Whitney's U test. The differences between groups that are presented in categorical variables after 3-months (end line) were compared using logistic regression after adjusting for age, gender and baseline values for folate, vitamin B-12, and vitamin D deficiencies. Deficiencies of folate, vitamin B-12, and vitamin D were defined as serum folate<15.9 nmol/L (7 ng/mL),[25] serum vitamin B-12 <170 pmol/L (230 pg/mL),[25] and serum 25-OHD-3 <20 ng/mL,[26] respectively. Data were analyzed according to an intention-to-treat principle and all significance tests were two-tailed. Values of *p* less than 0.05 were considered to be statistically significant.

RESULTS

The participant characteristics data at baseline were described in Table 2. The results showed no significant differences between the two groups (*p*>0.05). The dietary intakes at baseline and the end of the study were both described in Table 3. The dietary intakes of total energy, macronutrients and micronutrients at baseline were balanced between the two groups (all *p*>0.05). Energy and most nutrients intakes were also balanced between the two groups at the end of the study, except iron and vitamin B-12 in the intervention group were less than the control group. Total intake including dietary intakes and supplementation met the Chinese DRIs (> 80 years) in the intervention group (Figure 1).

Of the 98 subjects enrolled in this study, 16 subjects did not complete the study (Figure 2). The reasons for the dropouts included: withdrawal without reasons (n=8), diarrhea (n=4) and

discharged (n=4). Finally, 40 subjects in the intervention group and 42 subjects in the control group completed the study. Adherence to interventions was high (mean adherence, 99.3%±2.2% in intervention group vs 99.6%±1.4% in control group, p=0.511) in both the groups. The gastrointestinal intolerance was recorded in 2 subjects in the intervention group (one with dyspepsia and one with diarrhea). No other intervention-related serious adverse events occurred.

After 3-month intervention, the mean changes in serum folate, vitamin B-1, vitamin B-2, vitamin B-12, 25-OHD-3 and plasma zinc in the intervention group were significantly higher than those of the control group (Table 4). However, the changes in serum concentrations of iron, ferritin, magnesium, vitamin B-6, vitamin C and vitamin A were similar between the two groups.

After adjusting for age, gender and baseline prevalence, it was demonstrated that the 3-month intervention was able to markedly reduce the prevalence of folate deficiency by -92.5% (odd ratio (OR)=0.01, 95% confidence interval (CI): 0-0.30, p<0.001), vitamin B-12 deficiency by -15.0% (OR=0.09, 95% CI: 0.02-0.50, p<0.05), and vitamin D deficiency by -82.5% (OR=0.01, 95% CI: 0-0.04, p<0.001) when compared with that of the control group (-4.8%, +7.1% and +2.4%, respectively).

In addition, the antioxidative capacities were also enhanced in intervention group with changes in serum GSH-Px (1.3±0.3 vs 0.3±0.2 ng/mL, p=0.003) and plasma SOD (14.3±2.4 vs -2.1 ±2.4 U/mL, p<0.001) activities both increased, and changes in serum MDA (-1.5±0.2 vs 0.2±0.3 nmol/mL, p=0.001) concentrations were decreased when compared with those levels in control group (Figure 3).

DISCUSSION

The main purpose of this study was to assess the effect of micronutrient pack on the micronutrient status in institutional older adults. The results showed that the intake of this supplement reported good compliance and tolerance, assuring good efficacy through the intervention.

For institutional older adults, multiple factors, such as impaired mobility, poor oral health, loss of appetite, and alterations in cognition and vision can impose restrictions on the ability of older adults to access and consume nutrient-dense foods.[27] Therefore, even if a healthy diet enriched with micronutrients has been provided, the entire consumable amount of vitamins and minerals were still unable to meet the requirements of the Chinese reference

standard.[17,28,29] Hence, older adults were the targeted populations who were often overlooked for combating MND.

The dietary supplements of micronutrients have ramped up considerably in the past decades and widely used by older adults.[30] However, most of the supplements were in either a pill or capsule form that are designed to "reduce the risks of some chronic diseases when used by special populations."[30-32] In view of the potential harm of using the commercial supplements inappropriately, it is necessary to produce new supplement that complement the insufficient dietary intakes of micronutrients among older adults. As mentioned above, the supplementary approach of micronutrient pack has not been applied to older adults. Thus, in our study, the micronutrient pack as a complementary food-based supplement was produced for elderly, aiming to increase the total intake (including dietary intake and supplement of micronutrient) to DRIs.

As expected, after 3-month micronutrient intervention, serum folate, vitamin B-1, vitamin B-2, vitamin B-12, 25-hydroxyvitamin D and plasma zinc status were improved. While the folate, vitamin B-12, and vitamin D deficiencies were substantially decreased. Nonetheless, the concentrations of serum vitamin B-6, vitamin A, iron, ferritin and plasma magnesium showed no increase after supplementation. One possible reason for this could be that the dosage limit of micronutrients contained in a single package of micronutrient pack might not be sufficient. For example, there was only 4 mg iron, which accounted for only 27% (15 mg) of the recommended nutrient intake (RNI) in one package of micronutrient pack. The other explanation might be that the duration of intervention (3 months) might have been too short for investigating the trace elements regarding their turnover time. Thirdly, the reduction in the gastric acid secretion due to mucosal atrophy, bacterial-induced gastritis or the use of proton pump inhibitors might decrease the absorption efficiency in elderly persons.[33,34] In addition, the insensitivity of the selected assay methodology,[35] and the reduction of plasma carrier proteins[36] and drug interactions[37] might also show no improvements.

Our results also indicated that micronutrient supplementation could enhance geriatric antioxidative capacities in older adults. As a product of terminal degradation of lipids by lipid peroxidation, MDA indirectly reflected the radical attacks of lipids.[38] GSH-Px and SOD are the two most important antioxidant enzymes that participate in the transformation of hydrogen peroxide to water or elimination of superoxide, playing an important role in the antioxidative function. Similar to our results, supplementation with 50,000 IU vitamin D weekly for 8 weeks in type 2 diabetes and obesity patients could also increase the serum SOD activities (49.68±3.49 vs 51.31±4.34 U/ghb, $p<0.001$).[39] Treatment with vitamin B-2 could also

increase the activities of GSH-Px in CCl4-induced liver.[40] The possible explanation may be due to that: (1) the zinc and selenium of the dietary supplement essentially acted as cofactors for SOD and GSH-Px, respectively;[41,42] (2) Vitamin D might prevent ROS overproduction through the activation of vitamin D receptor by reducing the expression of non-phagocytic cell oxidase (NOX)-2 and NOX-4, and increasing the expression of SOD-1 genes;[43] (3) Some of the vitamins such as folate, vitamin B-6, and vitamin B-12 may take part in the chain of antioxidative reactions by donating and accepting the electrons from the reactive oxygen species (ROS), leading to the damage of DNA or other important cellular structures.[41] Future studies should investigate whether the physical functions in older adults may benefit from the increased antioxidative capacities.

However, the present study has some limitations. Firstly, this dietary supplement was designed based on the dietary survey of institutional older adults, and so whether it is applicable for free-living older adults remains to be validated. In addition, as medications, foods, and duration of intervention may also affect the absorption, larger population and longer periods are needed to confirm the effectiveness of this intervention.

In conclusion, the micronutrient pack was well-accepted among the institutional older adults with good compliance and tolerance. This complementary food-based supplement could improve the micronutrient status and benefit the blood antioxidant capacities among institutional older adults. Further research with larger population and longer periods is needed to verify the effectiveness and explore the optimal approach of the intervention.

AUTHOR DISCLOSURE

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Table 1. The characteristics of participating restaurants (n=90)

Composition	Micronutrient pack for elderly	Placebo	Chinese DRIs for adults >80 years
Energy (KJ)	244	244	1949/1535[†,‡]
Protein (g)	6.0	6.0	65/55[†,‡]
Fat (g)	0.4	0.4	-
Carbohydrate (g)	7.4	7.4	-
Calcium (mg)	500	-	1000[§]
Magnesium (mg)	100	-	310[§]
Zinc (mg)	7	-	12.5/7.5[†,‡]
Iron (mg)	4	-	12[§]
Selenium (µg)	26	-	60[‡]
Vitamin A (µg RAE)	200	-	800/700[†,‡]
Vitamin B-1 (mg)	1.4	-	1.4/1.2[†,‡]
Vitamin B-2 (mg)	1.4	-	1.4/1.2[†,‡]
Vitamin C (mg)	200	-	200[¶]
Vitamin D-3 (IU)	800	-	800[††]
Vitamin B-6 (mg)	1.1	-	1.6[§]
Vitamin B-12 (µg)	1.6	-	2.4[§]
Folate (µg DFE)	264	-	400[§]

[†]Male/Female.
[‡]Recommended nutritional intakes.
[§]Adequate intakes.
[¶]Proposed intake for preventing non-communicable chronic disease.
[††]Tolerable upper intake level.

Table 2. Baseline characteristics and blood micronutrient status of the subjects

	Control (n=49)	Intervention (n=49)	p value
Age (y)	83.0±8.5	84.6±5.6	0.279
<80 y	12 (55.1%)	5 (55.1%)	
≥80 y	37 (44.9%)	44 (44.9%)	
BMI (kg/m^2)	23.5±4.7	22.6±4.8	0.269
Gender (men/women)	18/31	23/26	0.306
Educational status (n)			0.082
None	8 (16.3%)	5 (10.2%)	
Primary	18 (36.7%)	32 (65.3%)	
Secondary or higher	23 (46.9%)	12 (24.5%)	
Diseases			
Hypertension	47/49 (95.9%)	44/49 (89.8%)	
Coronary heart disease	28/49 (57.1%)	23/49 (46.9%)	
Stroke	21/49 (42.9%)	24/49 (49.0%)	
Diabetes	14/49 (28.6%)	12/49 (24.5%)	
Hyperlipidemia	13/49 (26.5%)	9/49 (18.4%)	
Osteoporosis	11/49 (22.4%)	4/49 (8.2%)	
Dementia	4/49 (8.2%)	8/49 (16.3%)	
Gastritis	4/49 (8.2%)	8/49 (16.3%)	
Chronic bronchitis	3/49 (6.1%)	5/49 (10.2%)	
Cholecystitis	1/49 (2.0%)	7/49 (14.3%)	
MNA-SF score	9.6±1.5	9.0±1.8	0.116

BMI: body mass index; MNA-SF: mini nutritional assessment short-form.
Data are presented as means ± standard errors for continuous variables, and n (%) for categorical variables.
p value is defined as the difference assessed using independent sample Student's t test, Mann-Whitney's U test or Chi-square test.

Table 3. Dietary intakes of the subjects at baseline and the end of the study (3-month)

	Control			Intervention (n=49)			p_1 value	p_2 value
	Baseline n=49	3-month n=42	Mean changes† n=42	Baseline n=49	3-month n=40	Mean changes† n=40		
Total energy (kcal/d)	1574.6±52.1	1598.9±64.0	0.5±52.3	1504.9±39.6	1419.6±39.8	-73.0±35.5	0.289	0.253
Protein (g/d)	57.2±2.7	59.3±2.8	1.5±2.2	53.2±1.7	50.8±1.4	-2.8±1.6	0.216	0.113
Fat (g/d)	56.0±2.8	60.4±3.4	4.4±2.5	51.5±1.9	49.4±1.6	-1.5±1.8	0.185	0.057
Carbohydrate (g/d)	210.5±7.8	196.4±9.9	-16.4±7.2	207.1±7.0	193.1±7.4	-12.0±6.0	0.749	0.644
Calcium (mg/d)	325.8±19.1	345.3±20.3	22.9±22.7	379.6±27.8	352.6±21.9	-20.2±24.6	0.114	0.201
Iron (mg/d)	11.0±0.5	11.0±0.6	-0.2±0.4	11.3±0.6	9.8±0.5	-1.7±0.4	0.656	0.009**
Zinc (mg/d)	7.9±0.3	8.2±0.3	0.2±0.3	7.8±0.3	7.5±0.4	-0.3±0.3	0.746	0.177
Selenium (μg/d)	38.4±3.4	40.3±3.1	0.03±2.35	34.7±2.6	30.1±1.7	-5.7±2.6	0.381	0.103
Magnesium (mg/d)	196.9±7.3	187.0±8.1	-8.8±6.1	200.2±7.9	176.7±7.2	-25.1±5.6	0.758	0.053
Vitamin A (μg RAE/d)	356.1±34.0	447.0±45.6	89.3±25.8	383.0±42.5	376.9±31.0	18.6±30.8	0.622	0.081
Vitamin B-1 (mg/d)	0.9±0.03	0.8±0.03	-0.05±0.04	0.9±0.05	0.8±0.05	-0.04±0.03	0.804	0.953
Vitamin B-2 (mg/d)	0.7±0.03	0.7±0.04	0.05±0.03	0.7±0.06	0.7±0.05	-0.06±0.04	0.314	0.039*
Vitamin C (mg/d)	61.7±5.0	54.2±4.2	-9.4±3.7	59.9±6.0	48.6±4.9	-11.8±4.2	0.818	0.669

Data are presented as mean ± standard deviations.

†Mean changes were calculated by subtracting the 3-month values from the baseline values of each group.

p_1 value is the differences of dietary intakes between two groups at baseline, p_2 value is the differences of changes in dietary intakes between two groups. Both p values are assessed using independent sample Student's t tests or Mann-Whitney's U test, * $p<0.05$, ** $p<0.01$.

Table 4. Blood micronutrient concentrations at baseline and the end of the study (3-month)

	Control			Intervention			p_1 value	p_2 value
	Baseline (N=49)	3 months (n=42)	Mean changes† (n=42)	Baseline (n=49)	3 months (n=40)	Mean changes† (n=40)		
Serum folate (nmol/L)	6.4±0.4	6.7±0.7	0.6±0.5	6.7±0.3	28.3±1.6	21.1±1.6	0.615	<0.001**
<80 y			2.3±1.1 (n=12)			17.4±2.8 (n=5)		<0.001**
≥80 y			-0.02±0.5 (n=30)			21.6±1.7 (n=35)		<0.001**
Serum vitamin B-1 (nmol/L)	5.6±0.3	5.4±0.3	-0.2±0.3	5.0±0.3	8.4±0.5	3.4±0.4	0.130	<0.001**
<80 y			-0.01±0.7 (n=12)			4.7±1.5 (n=5)		0.005**
≥80 y			-0.3±0.4 (n=30)			3.2±0.4 (n=35)		<0.001**
Serum vitamin B-2 (nmol/L)	24.1±1.9	25.7±2.3	2.3±1.4	26.4±2.1	36.4±4.0	11.5±3.3	0.427	0.014*
<80 y			5.1±3.4 (n=12)			1.0±4.0 (n=5)		0.504
≥80 y			1.2±1.5 (n=30)			13.0±3.7 (n=35)		0.005**
Serum vitamin B-6 (nmol/L)	58.1±3.8	69.6±6.4	10.8±5.7	51.5±3.6	72.5±4.9	17.9±4.9	0.208	0.353
<80 y			11.0±8.7 (n=12)			40.9±5.7 (n=5)		0.053
≥80 y			10.7±7.3 (n=30)			14.6±5.4 (n=35)		0.665
Serum vitamin B-12 (pmol/L)	315.4±31.2	332.7±39.8	13.3±16.0	279.7±21.3	413.7±41.2	128.8±34.8	0.986	0.001**
<80 y			36.6±34.6 (n=12)			84.4±54.3 (n=5)		0.459
≥80 y			4.0±17.7 (n=30)			135.1±39.2 (n=35)		0.004**
Serum vitamin C (μmol/L)	70.9±2.5	75.1±3.1	5.0±4.0	64.7±3.2	73.5±2.5	9.4±3.7	0.062	0.419
<80 y			-5.3±6.8 (n=12)			-4.3±8.5 (n=5)		0.934
≥80 y			9.1±4.8 (n=30)			11.4±3.9 (n=35)		0.714
Serum vitamin A (μmol/L)	190.0±10.9	190.3±8.9	-1.5±15.4	164.1±8.3	190.2±15.7	26.7±16.4	0.131	0.214
<80 y			-3.3±31.9 (n=12)			57.2±58.3 (n=5)		0.343
≥80 y			-0.8±17.8 (n=30)			22.3±17.1 (n=35)		0.353
Serum 25-OHD-3 (ng/mL)	13.2± 0.8	12.4±0.8	-0.8±0.5	11.4±0.7	29.4±1.3	17.8±1.3	0.109	<0.001**
<80 y			-0.2±0.8 (n=12)			19.6±1.6 (n=5)		<0.001**
≥80 y			-1.0±0.7 (n=30)			17.6±1.4 (n=35)		<0.001**
Serum iron (μmol/L)	12.8±0.4	14.3±0.7	1.4±0.7	14.0±0.6	14.9±0.8	1.1±0.8	0.149	0.817
<80 y			0.9±1.6 (n=12)			-0.7±1.5 (n=5)		0.585
≥80 y			1.6±0.8 (n=30)			1.4±0.9 (n=35)		0.863
Serum ferritin (ng/mL)	186.2 ±17.9	189.4±20.2	0.8±11.7	173.5±18.0	174.8±19.7	18.6±13.6	0.579	0.204
<80 y			21.1±28.6 (n=12)			-31.6±49.5 (n=5)		0.348
≥80 y			-7.3±11.8 (n=30)			25.8±13.7 (n=35)		0.077
Plasma zinc (umol/L)	87.8±2.0	78.9±1.7	-9.6±1.9	87.2±1.5	88.9±2.3	0.6±1.8	0.814	<0.001**
<80 y			-6.9±5.2 (n=12)			-2.5±3.4 (n=5)		0.608
≥80 y			-10.7±1.8 (n=30)			1.1±2.0 (n=35)		<0.001**
Plasma magnesium (mmol/L)	1.3±0.1	1.2±0.02	-0.04±0.02	1.3±0.1	1.3±0.02	-0.001± 0.017	0.554	0.157
<80 y			-0.02±0.05 (n=12)			0.01±0.03 (n=5)		0.708
≥80 y			-0.04±0.02 (n=30)			0.01±0.02 (n=35)		0.124

Data are expressed as mean±standard deviations. †Mean changes were calculated by subtracting the 3-month values from the baseline values of each group. p_1 value is the differences of blood micronutrient concentrations between two groups at baseline, p_2 value is the differences of changes in blood micronutrient concentrations between two groups. Both p values are assessed using independent sample Student's t tests or Mann-Whitney's U test, *$p<0.05$, **$p<0.01$.
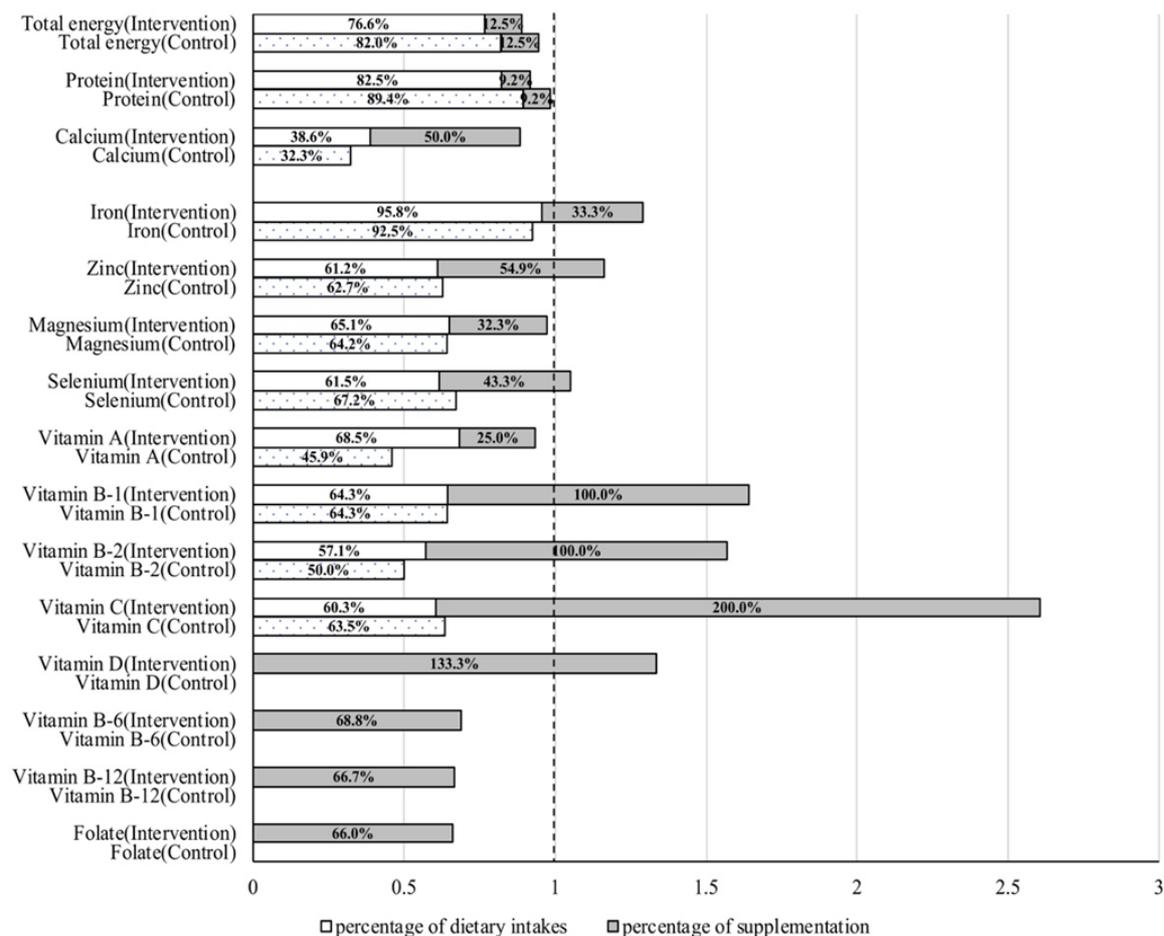
Figure 1. The total intake including dietary intakes and supplementation. The percentage of dietary intakes were calculated by using the formula of (dietary intakes)/(Chinese DRIs for men > 80 years) × 100%. The dash line represents 1×DRIs. As the dietary intakes of vitamin B-6, vitamin B-12, vitamin D and folate were not included in China's food composition tables, so they were not shown in the figure.
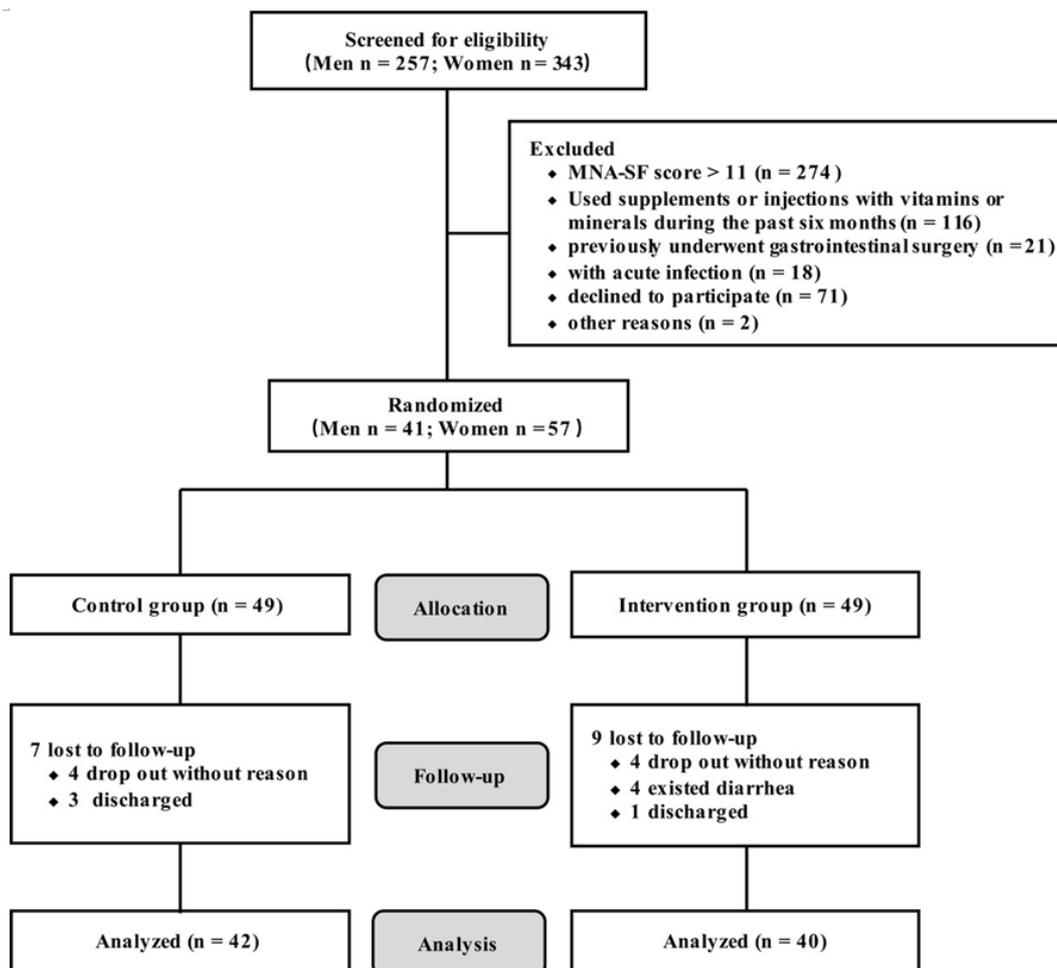
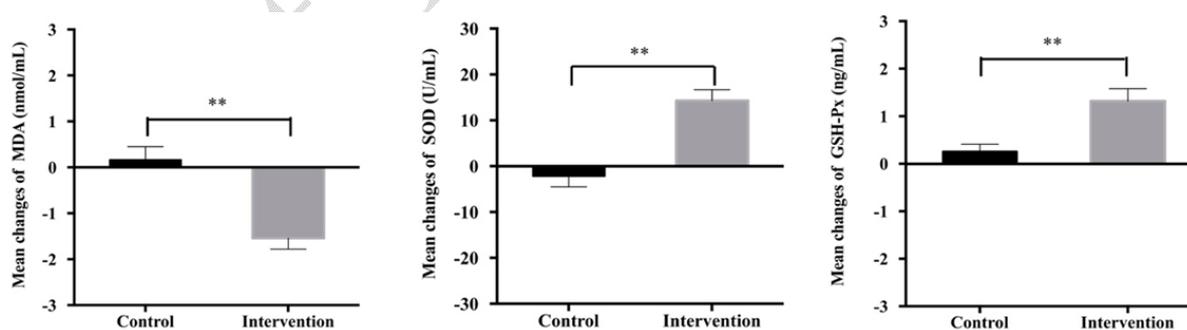**Figure 2.** Flow diagram of participants through the study.

Figure 3. Mean changes (3-month - baseline) of blood oxidative/antioxidative biomarkers between the intervention group (n=40) and the control group (n=42). MDA: malondialdehyde; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase. Data are presented as means±standard errors. Differences in the changes between the two groups was assessed by independent samples Student's t test or Mann-Whitney's U test. **p<0.01.