Original Article

Effects of supplementation with multivitamin and mineral on blood pressure and C-reactive protein in obese Chinese women with increased cardiovascular disease risk

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Objective: To investigate the effect of supplementation with multivitamin and mineral on blood pressure and C-reactive protein (CRP) in obese women with increased cardiovascular disease risk as having hypertension, hyperglycemia or hyperlipemia. Subjects and Methods: 128 obese Chinese women aged 18-55 years with increased cardiovascular disease risk participated in a 26-week randomized, double-blind, placebo-controlled trial. Subjects were randomized to four groups, and received either one tablet of high-dose multivitamin and mineral supplement (MMS), or one tablet of low-dose MMS (Low MMS), or calcium 162 mg (Calcium) or identical placebo (Placebo) daily during the study. Diastolic blood pressure (DBP), systolic blood pressure (SBP) and serum concentrations of CRP were measured at baseline and end-trial. Results: At baseline, the subjects had an average age of 42.0±7.1 years and BMI of 30.9±2.8 kg/m^2. There were no significant differences between the four groups in baseline characteristics. One hundred and seventeen subjects completed the study. After 26-week supplementation, both SBP and DBP were significantly lower in the MMS group compared to the placebo group ($p < 0.05$). There was also a non-significant trend of lower DBP at 26-week in the MMS and calcium groups compared to baseline ($p < 0.08$). At 26-week, the MMS group also had significantly lower serum concentrations of CRP compared with that of baseline and the placebo group ($p < 0.05$). Conclusions: Our results showed that supplementation with adequate multivitamin and mineral supplement could reduce blood pressure and serum CRP in obese women with increased cardiovascular disease risk.

Key Words: obesity, blood pressure, C-reactive protein, multivitamin and mineral supplementation, Chinese women

INTRODUCTION

The World Health Organization (WHO) estimates that there are more than 1 billion overweight adults worldwide, and 300 million of them are obese.[1] In China, according to the 2002 National Nutrition Survey, 22.8% of adults are overweight and 7.1% obese.[2] It has been reported that obese and overweight people are at higher risks of hypertension and cardiovascular diseases than people with normal body weight.[1-5] Therefore, it is important to find effective ways to prevent and control hypertension in people with obesity.

As pharmaceutical treatment of hypertension has attendant risks due to its adverse effects,[6, 7] non-pharmaceuti-cal treatment of hypertension has received a lot of interest recently. A number of studies have been carried out to evaluate the effects of supplementation with vitamins and/or minerals on blood pressure in general population.[8-14] Whereas some studies have shown effects on reducing blood pressure,[8,9] some failed to show such effect.[10-14] This may be due to the differences in study population and the supplement used. Obese individuals are more likely to have either lower blood concentrations or lower bioavailability of minerals and/or vitamins to some degrees.[15-18] However, the effects of vitamin and mineral supplementation on blood pressure have not been studied in obese people. In addition, most of the previous

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studies have only evaluated the effects of supplementation with several kinds of minerals and/or minerals on blood pressure, whereas a number of minerals and vitamins including calcium, iron, zinc, magnesium, thiamin, vitamin D play important roles in the regulation of blood pressure.[19-22] In order to prevent and control hypertension, obese individuals may need to be supplemented with all these vitamins and minerals instead of only a selective few.

To date, a large number of studies have suggested that obesity is associated with chronic low-grade inflammation,[23] and chronic inflammation plays an important role in the pathogenesis of metabolic syndrome and hypertension.[24,25] It is well-recognized that C-reactive protein (CRP), a marker of systemic inflammation, is an independent predictor of metabolic syndrome and hypertension.[26-28] The aim of this study is to evaluate the effect of supplementation with multivitamin and mineral on blood pressure and CRP in obese Chinese women with increased cardiovascular disease risk as having hypertension, hyperglycemia or hyperlipemia.

SUBJECTS AND METHODS

Subjects

Two hundred and eighty-three obese women, aged between 18 to 50 years were recruited from communities of Harbin, Heilongjiang Province in the North of China based on body mass index (BMI) above 28 kg/m^2.[29] Among them, 128 women who met the inclusion criteria participated in this 26-week randomized, double-blind, placebo-controlled trial. The inclusion criteria was as the followings: 1) hypertension or/and hyperglycemia and/or hyperlipemia, 2) stable body weight during the 6 months before the study, 3) less than 2 h/week of regular physical activity, 4) no vitamin and mineral supplements were used in the 6 months before the beginning of the study, 5) no history of myocardial infarction and diabetes and not pregnant. The subjects were fully informed of the purpose, procedures and hazards of the trial and were free to withdraw from the trial at any time. The study was approved by the Ethics Committee of the Harbin Medical University, and an informed consent form was signed by all participants. All investigators were trained by researchers or medical personnel from the Harbin Medical University, P. R. China.

Intervention

The study subjects were randomly assigned to four treatment groups stratified by blood pressure, BMI and the levels of blood glucose and blood lipid. There were 32 subjects in each group and subjects in the multivitamin and mineral supplement group (MMS group) received one tablet of high-dose multivitamin and mineral supplement as detailed in Table 1, subjects in the low MMS group received one tablet of low-dose multivitamin and mineral supplement (half the amount of the high-dose tablet), subjects in the calcium group received 162 mg calcium and those in the placebo group received identical placebo composed of maize starch daily during the 26 weeks study period. The commercially available multivitamin and mineral supplement was obtained from Wyeth Pharmaceutical Co., LTD (29-ingredient multivitamin and mineral formula, Harbin, China) and calcium carbonate which contained the same amount of calcium as the supplement for the MMS group (162 mg), was purchased from the SHANGHAI DA YU BIOCHEMISTRY CO., LTD (Shanghai, China).

The randomisation schedule for the four groups was generated from a randomisation number table by an independent research scientist, who also labelled the bottles and dispensed the study medications to subjects. The study subjects and the study staff remained blinded to the treatment code until all the data has been entered, evaluated for accuracy and the a-priori hypotheses reviewed.

Venous blood samples after overnight fasting, dietary intakes and demographic and clinical data were collected at baseline and 26 weeks. During the study, subjects were followed up once every two months for the distribution of treatment tablets. Compliance with the supplementation was checked at the same time by counting tablets returned.

Blood pressure

Blood pressure was measured using a standard mercury sphygmomanometer on the right arm after at least 10 minutes of rest. Mean values were determined from two independent measurements (by the same researcher) at two-minute intervals. Subjects were classified as having

Table 1. Composition of the multivitamin and mineral supplement used in the MMS group

Ingredient	Amount	% RDA
Vitamin A(containing natural mixed beta carotene)	5000 IU	214
Vitamin D	400 IU	200
Vitamin E	30 IU	142
Thiamin	1.5 mg	115
Riboflavin	1.7 mg	142
Vitamin B6	2 mg	167
Vitamin C	60 mg	60
Vitamin B12	6 μg	250
Vitamin K_1	25 μg	*
Biotin	30 μg	100
Folic acid	400 μg	100
Micotinamide	20 mg	154
Pantothenic acid	10 mg	200
Calcium	162 mg	20
Phosphorus	125 mg	18
Chlorine	36.3 mg	*
Magnesium	100 mg	28
Iron	18 mg	90
Copper	2 mg	100
Zinc	15 mg	100
Manganese	2.5 mg	71
Iodine	150 μg	100
Chromium	25 μg	50
Molybdenum	25 μg	42
Selenium	25 μg	50
Nickel	5 μg	*
Stannum	10 μg	*
Silicon	10 μg	*
Vanadium	10 μg	*

* RDA value for Chinese women has not been established.

hypertension if they had a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg according to the current ESH/ESC (European Society of Hypertension/European Society of Cardiology) hypertension guidelines.[30]

Dietary intake

Dietary intakes were determined using a validated semi-quantitative food-frequency questionnaire (FFQ). The FFQ was designed according to the method of Willett [31] and the dietary habit of local inhabitants and has been validated by a one-week dietary record. The FFQ contained 103 items, including food intakes, alcohol drinks and multivitamin and mineral and calcium supplements. The intakes of energy and 25 nutrients were calculated using the Food Nutrition Calculator (V1.60; Chinese CDC nutrition and food security institute).

Demographic

The following demographic data were collected using a standardized questionnaire: age, nationality, education, occupation, smoking habits, physical activity, health history and medication use. Physical activity included the activity during the leisure time and at work. The level of physical activity during leisure time (PAL) was defined as: 0 = no exercise, 1 = 0~30 min/week, 2 = 30~60 min/week, 3 = 60~90 min/week, 4 = 90~120 min/week and 5 ≥ 120 min /week. The level of physical activity at work (PAW) was defined as: 1 = sedentary, 2 = moderate, and 3 = heavy, based on the definition of physical activity in Chinese women.[32]

Anthropometry

Body weight and height were measured after the subjects fasted overnight and were wearing only underwear. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (meters). Subjects with BMI ≥ 28 kg/m^2 were categorized as obese according to the criteria for the Chinese population.[29]

Biochemistry

Venous blood samples were collected after overnight fasting for the measurement of serum concentrations of CRP, calcium, iron, zinc, thiamin and riboflavin and plasma concentrations of glucose, and were collected again 2 hours after taking 75 g glucose for the measurement of plasma concentration of glucose for the oral glucose tolerance test. Plasma glucose was measured by Kyoto blood sugar test meter and test strip. Serum total cholesterol, and triglycerides were assessed with standard enzymatic spectrophotometric techniques. Hyperlipemia was defined as a total cholesterol (TC) level > 5.72 mmol/L, and/ or a triglyceride level (TG) ≥ 1.70 mmol/L (Hypertriglyceridemia). Hyperglycemia was defined as fasting blood glucose concentration between 6.1-7.0 mmol/L and/or 2-h blood glucose concentration of between 7.8 - 11.0 mmol/L. High-sensitivity CRP was measured by quantitative sandwich enzyme immunoassay assay (Quantikine Human C-Reactive Protein Immunoassay, R&D Systems Inc., Minneapolis, U.S.A), and the intra- and interassay coefficients of variation (CV) were 3.8% and 7.0%, respectively. Serum iron was measured with ferro-zin chromatometry using commercial kit (Shanghai Iatrology Ltd). Serum calcium and zinc were measured with complexation chromatometry using commercial kits (Ningbo Asia-Pacific Biotechnology Ltd). Serum thiamin and riboflavin concentrations were measured using reversed phase HPLC with a photodiode array detector (model 2695 and 996, respectively, Waters Technologies, Massachusetts, U.S.A).

Power calculation and statistical analysis

Power calculations were performed prior to the commencement of the study. A sample size of 28 in each group would be sufficient to detect a difference of 7.5 mmHg in DBP between the treatment and the placebo groups assuming a standard deviation of 10 mmHg as reported in this population, at 80% power and 5% level of significance. This number has been increased to 32 per group (total of 128) to allow for a predicted drop-out from treatment of around 10%.

Statistical analysis in this study was performed using the Statistical Package of Social Sciences (SPSS) 13.0 (version 13.01S; Beijing Stats Data Mining Co. Ltd). Results are presented as mean ± standard deviation (SD) or M-estimators by Andrew with inter-quartile range as appropriate. Data that were not normally distributed, as determined with the Kolmogorov-Smirnov test, were logarithmically transformed to obtain near normality before analysis. In this study, baseline characteristics between the four groups were compared using analysis of variance (ANOVA) with Tukey's post hoc test, Chi-square test and Kruskal-Wallis H test where appropriate. Paired t-tests were used to evaluate the changes in outcome variables before and after intervention in each group. The differences in outcome variables between groups after intervention were evaluated by analyses of covariance (ANCOVA), adjusted for baseline values and other confounding factors including age, alcohol consumption, smoking habits, physical activity at work and at leisure time and menopause. All p values are 2-tailed and $p < 0.05$ was considered as significant for all statistical analysis in this study.

RESULTS

Subjects retention and compliance

Among the 128 eligible individuals who participated in the study, 11 participants withdrew during the study due to employment commitments (n = 2), lost of follow-up (n = 5), change of residence (n = 2), pregnant (n = 1) and illness (n = 1) (Figure 1). There were no significant differences between the four groups in the percentage of subjects who withdrew from the study and the overall compliance rate to the assigned treatment (Placebo 92.6%, MMS 94.9%, low MMS 93.5%, Calcium 93.7%, p = 0.71).

Baseline characteristics

At baseline, there were no significant differences between the four groups in age, weight, height, BMI, daily physical activity level at leisure time and at work, the percentage of subjects who are currently smoking, consuming alcohol or had reached menopause, the percentage of subjects with hyperglycemia, hyperlipidemia or hypertension,

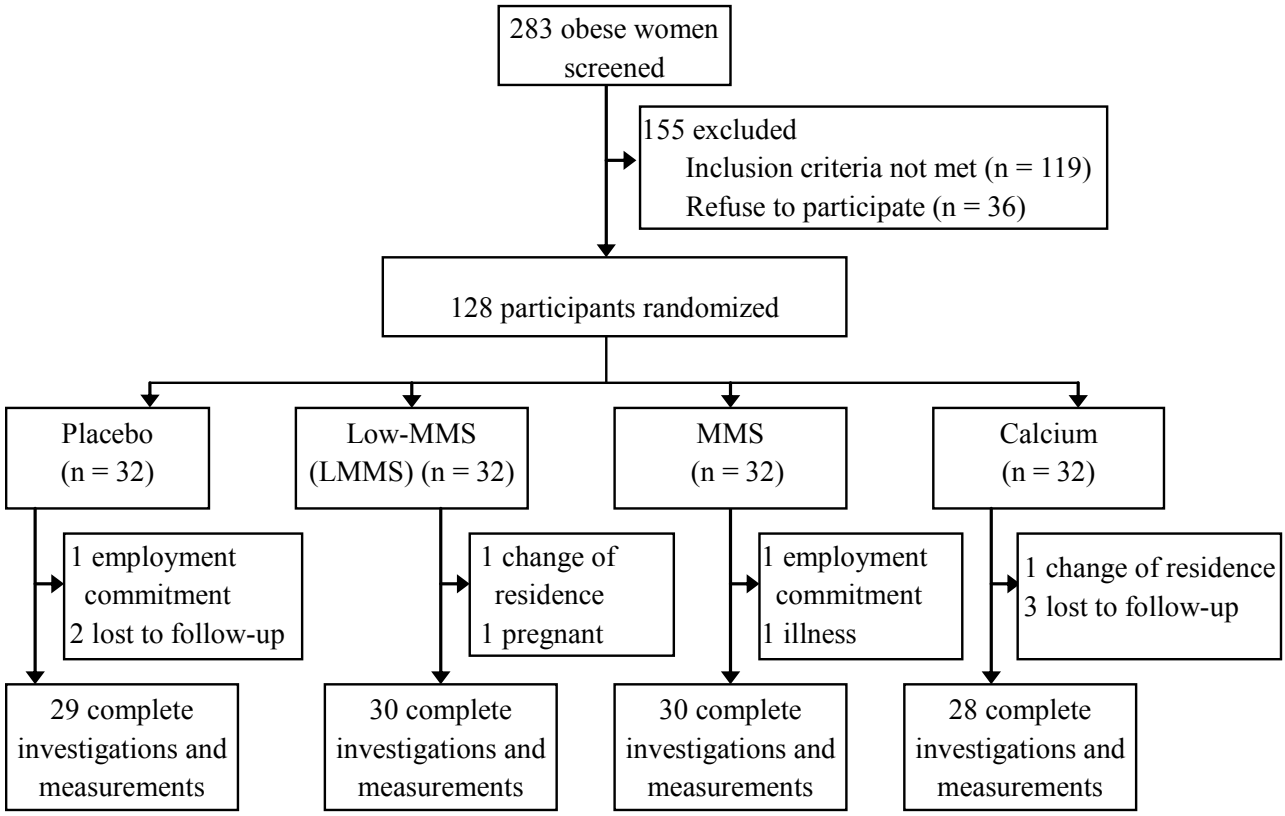

Figure 1. The study design and the flow of subjects

Table 2. Characteristics of subjects at baseline

	Placebo (n = 29)	LMMS (n = 30)	MMS (n = 30)	Calcium (n = 28)	$p^{\dagger}$
Age (years)	41.2 ± 6.8	42.5 ± 7.1	42.8 ± 6.9	41.6 ± 9.0	0.82
Weight (kg)	80.8 ± 9.7	76.8 ± 8.1	80.5 ± 8.0	77.5 ± 8.0	0.17
Height (cm)	161.0 ± 5.2	158.6 ± 5.3	161.6 ± 5.5	159.3 ± 4.5	0.09
BMI (kg/m^2)	31.1 ± 2.7	30.5 ± 2.4	30.8 ± 2.5	30.5 ± 2.5	0.87
Physical activity at leisure time (%)					
No exercise	79.3	83.3	80.0	78.6	0.51
0-30 minutes/week	10.3	16.7	10.0	14.4	
30-60 minutes/week	3.4		10.0	3.5	
60-120 minutes/week	7.0			3.5	
Physical activity at work (%)					
Sedentary	69.0	73.3	86.7	78.5	0.36
Moderate	31.0	26.7	13.3	17.9	
Heavy	0			3.6	
Current smoking (%)	6.9	10.0	10.0	3.6	0.77
Consume alcohol (%)	27.6	40.0	46.7	21.4	0.16
Menopause (%)	24.1	33.3	20.0	28.6	0.35
Hyperglycemia (%)	39.3	60.0	43.3	46.4	0.41
Hyperlipidemia (%)	75.9	66.7	70.0	67.9	0.87
Hypertension (%)	44.8	46.7	50.0	42.9	0.95
Systolic blood pressure (mmHg)	129.5 ± 26.7	131.7 ± 27.4	126.5 ± 18.4	129.0 ± 15.3	0.85
Diastolic blood pressure (mmHg)	84.7 ± 13.3	84.4 ± 13.8	85.2 ± 15.0	86.4 ± 11.7	0.66

Values are mean ± SD unless otherwise stated. BMI, body mass index, LMMS, low multivitamin and mineral supplementation group, received one tablet of low-dose multivitamin and mineral supplement daily during the study; MMS, multivitamin and mineral supplementation group, received one tablet of high-dose multivitamin and mineral supplement daily during the study.
†ANOVA for continuous variables and X^2 test or Kruskal-Wallis H test for categorical variables.

Table 3. Dietary intakes at baseline

	Placebo (n = 29)	LMMS (n = 30)	MMS (n = 30)	Calcium (n = 28)	*p*
Energy (kcal/d)	2841.3 ± 797.4	2767.3 ± 839.9	2765.8 ± 950.2	2567.9 ± 686.9	0.57
Protein (g/d)	88.9 ± 28.5	88.2 ± 32.5	83.5 ± 28.4	78.6 ± 29.4	0.53
Fat (g/d)	94.8 ± 32.6	96.7 ± 33.5	98.1 ± 43.1	94.7 ± 37.2	0.98
Carbohydrate (g/d)	428.7 ± 151.5	411.3 ± 140.0	408.3 ± 151.6	369.9 ± 104.7	0.45
Fiber (g/d)	22 .3 ± 10.7	26.9 ± 15.1	22.9 ± 15.2	21.4 ± 10.5	0.31
Vitamin A (μg/d) †	785.2 (394.0-1371.5)	733.3 (372.3-1176.5)	598.4 (388.3-881.5)	575.4 (307.3-980.8)	0.39
Thiamin (mg/d)	1.3 ± 0.4	1.2 ± 0.5	1.3 ± 0.5	1.1 ± 0.4	0.57
Riboflavin (mg/d)	1.3 ± 0.5	1.3 ± 0.6	1.2 ± 0.5	1.2 ± 0.6	0.78
Vitamin C (mg/d)	150.6 ± 70.2	188.2 ± 104.3	163.8 ± 87.6	140.7 ± 89.5	0.26
Niacin (mg/d)	20.5 ± 7.8	19.2 ± 7.8	18.9 ± 7.1	17.2 ± 8.5	0.47
Total Vitamin E (mg/d)	54.4 ± 15.2	61.1 ± 20.6	63.9 ± 21.2	61.4 ± 21.0	0.30
Calcium (mg/d)	643.4 ± 299.8	739.0 ± 373.6	617.0 ± 289.9	636.6 ± 324.8	0.47
Magnesium(mg/d)	450.2 ± 155.4	500.6 ± 216.0	472.6 ± 184.8	429.4 ± 165.8	0.50
Iron (mg/d) †	48.9 (29.2-75.7)	49.4 (34.4-75.7)	41.7 (31.3-58.8)	42.34 (27.8-67.8)	0.69
Phosphorus (mg/d)	1400.9 ± 434.1	1450.9 ± 575.6	1371.4 ± 499.0	1302.9 ± 486.5	0.73
Selenium (μg/d)	58.3 ± 22.2	53.4 ± 23.8	52.6 ± 19.9	48.1 ± 27.6	0.44
Potassium (mg/d)	2673.9 ± 903.8	2873.9 ± 1245.6	2677.8 ± 1025.1	2435.8 ±1096.1	0.50
Total sodium (g/d)	9.7 ± 3.3	10.1 ± 3.9	8.6 ± 3.7	9.0 ± 4.3	0.44
Zinc (mg/d)	15.8 ± 5.0	15.9 ± 5.6	15.3 ± 5.4	14.7 ± 5.2	0.80
Copper (mg/d)	3.6 ± 1.3	4.0 ± 1.8	3.7 ± 1.5	3.4 ± 1.4	0.48
Manganese (mg/d)	10.9 ± 4.7	11.7 ± 5.4	10.1 ± 3.9	10.0 ± 4.4	0.46

Values are mean ± SD unless otherwise stated. LMMS, low multivitamin and mineral supplementation group, received one tablet of low-dose multivitamin and mineral supplement daily during the study; MMS, multivitamin and mineral supplementation group, received one tablet of high-dose multivitamin and mineral supplement daily during the study.
† Log transformed before analysis, and data were expressed M-estimators by Andrew and inter-quartile range (P_{25}-P_{75}).

and systolic and diastolic blood pressure (Table 2). There were also no significant differences between the four groups in dietary nutrients intakes (Table 3) and serum concentrations of high-sensitivity C-reactive protein, calcium, iron, zinc, thiamin and riboflavin at baseline (Table 4).

Effects on blood pressure (Figure 2)

After 26 weeks of supplementation, SBP was significantly lower in the MMS group in comparison to the placebo group (121.8 ± 13.7 mmHg vs. 130.8 ± 22.8 mmHg, $p = 0.046$). Diastolic blood pressure was also significantly lower in the MMS group in comparison to the placebo group (80.0 ± 8.7 mmHg vs. 85.7 ± 10.7 mmHg, $p = 0.008$) at trial-end (Figure 2). There were no significant changes in SBP and DBP from baseline to the end-point in each group. However, there was a non-significant trend of lower DBP in MMS and calcium groups at trial-end in

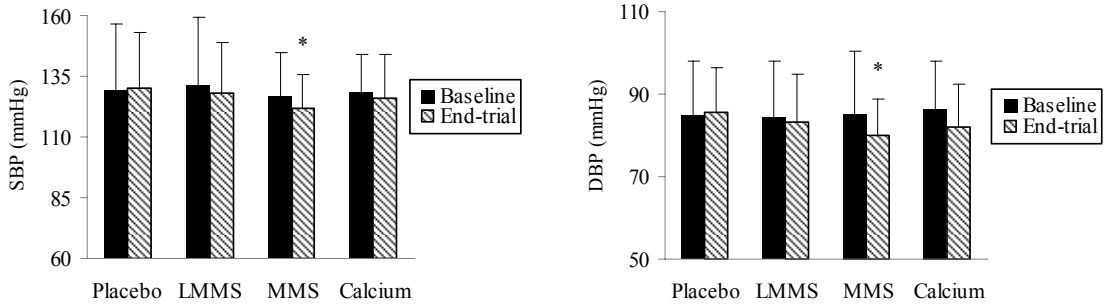

Figure 2. Unadjusted levels of diastolic blood pressure (DBP) and systolic blood pressure (SBP) before and after 26-week multivitamin and mineral or calcium supplementation in obese women. LMMS, low multivitamin and mineral supplementation group, received one tablet of low-dose multivitamin and mineral supplement daily during the study; MMS, multivitamin and mineral supplementation group, received one tablet of high-dose multivitamin and mineral supplement during the study. There were no significant changes from baseline to 26 weeks in each group. * $p < 0.05$ compared with the placebo group (ANCOVA adjusted for baseline values, age, alcohol consumption, smoking habits, total physical activity at leisure time and at work and menopause).

Table 4. Levels of serum C-reactive protein, calcium, iron, zinc, thiamin and riboflavin in obese women before and after 26-week multivitamin and mineral or calcium supplementation

	Placebo (n = 29)	LMMS (n = 30)	MMS (n = 30)	Calcium (n = 28)
hs-CRP (mg/L)				
Baseline	6.8 ± 3.6	6.4 ± 3.3	6.7 ± 3.2	6.9 ± 3.5
End-trial	7.0 ± 3.3	6.1 ± 2.2	5.8 ± 2.7*,†	6.6 ± 3.1
Serum Calcium (mmol/L)				
Baseline	2.33 ± 0.17	2.31 ± 0.15	2.32 ± 0.15	2.30 ± 0.13
End-trial	2.31 ± 0.14	2.37 ± 0.12	2.43 ± 0.14*,†	2.41 ± 0.12*,†
Serum Iron (μmol/L)				
Baseline	16.7 ± 6.2	17.3 ± 6.2	16.2 ± 6.4	17.9 ± 7.3
End-trial	17.3 ± 5.1	19.2 ± 5.4	18.5 ± 5.2*	16.9 ± 6.1
Serum Zinc (μmol/L)				
Baseline	23.1 ± 5.9	22.2 ± 6.5	22.4 ± 6.2	22.4 ± 6.76
End-trial	22.5 ± 5.2	24.2 ± 5.8	24.9 ± 5.1*,†	22.2 ± 5.1
Thiamin (μg/L)				
Baseline	9.6 ± 3.3	9.4 ± 3.1	9.7 ± 3.4	9.9 ± 3.6
End-trial	9.3 ± 3.1	10.2 ± 2.9	10.6 ± 3.5 *,†	9.8 ± 3.5
Riboflavin (μg/L)				
Baseline	7.4 ± 2.9	7.5 ± 3.3	7.2 ± 3.1	7.2 ± 2.8
End-trial	7.5 ± 3.2	8.3 ± 3.0 *	8.5 ± 3.4 *,†,‡	7.4 ± 2.6

Values are mean ± SD, and the comparisons between groups (p values) at the trial-end were adjusted for baseline values and other confounding factors including age, alcohol consumption, smoking habits, physical activity at work and at leisure time and menopause. LMMS, low multivitamin and mineral supplementation group, received one tablet of low-dose multivitamin and mineral supplement daily during the study; MMS, multivitamin and mineral supplementation group, received one tablet of high-dose multivitamin and mineral supplement during the study. There were no significant baseline differences between groups.

* Significant difference compared to that of baseline, $p < 0.05$.

† Significant difference compared to that of the Placebo group, $p < 0.05$.

‡ Significant difference compared to that of the Calcium group, $p < 0.05$.

comparison to baseline (MMS, 85.2 ± 15.2 mmHg vs. 80.0 ± 8.7 mmHg, $p = 0.059$; calcium, 86.4 ± 11.7 vs. 82.2 ± 10.3 mmHg, $p = 0.072$).

Effects on serum concentrations of high-sensitivity C-reactive protein

After 26 weeks of supplementation, the MMS group had significantly lower serum concentrations of hs-CRP compared with that of baseline ($p = 0.001$). There were no significant changes from baseline to trial-end in the other groups. At trial-end, the MMS group also had significantly lower serum levels of hs-CRP compared with the placebo group ($p = 0.005$) and a non-significant trend of lower serum hs-CRP level compared with the low MMS group ($p = 0.081$). (Table 4)

Effects supplementation on serum concentrations of calcium, zinc and iron

After 26 weeks of supplementation, the MMS group had significantly higher serum concentrations of calcium, iron and zinc compared with baseline (calcium, $p = 0.009$; iron, $p = 0.015$; zinc, $p = 0.029$). At trial-end, the MMS group also had significantly higher levels of serum calcium and zinc compared with the placebo group (calcium, $p = 0.005$; zinc, $p = 0.046$) and a non-significant trend of higher serum zinc level compared with the calcium group ($p = 0.069$). In the low MMS group, there was a non-significant trend of higher serum calcium concentration at trial-end in comparison to baseline ($p = 0.096$). At trial-end, the calcium group had significantly higher levels of serum calcium in comparison to that of baseline ($p = 0.004$) and the placebo group ($p = 0.01$). There were no significant changes from baseline to trial-end in the placebo group in serum calcium, zinc and iron. (Table 4)

Effects on thiamin and riboflavin At trial-end, the MMS group had significantly higher levels of serum thiamin and riboflavin compared to that of baseline (thiamin, $p = 0.008$; riboflavin, $p = 0.005$) and the placebo group (thiamin, $p = 0.039$; riboflavin, $p = 0.025$) and significantly higher riboflavin compared to the calcium group ($p = 0.022$). The LMMS group had significantly higher riboflavin ($p = 0.030$) and a non-significant trend of higher thiamin ($p = 0.078$) at end-trial compared to that of baseline. There were no significant changes in thiamin and riboflavin from baseline to trial-end in the placebo and calcium groups. (Table 4)

There were no significant differences between the four groups in dietary nutrients intakes during the intervention period, and there were no significant changes in dietary

nutrients intakes from baseline to trial-end in each group (data not shown).

DISCUSSION

Our study showed that 26-week multivitamin and mineral supplementation in full dose could significantly reduce both systolic and diastolic blood pressure and serum CRP in obese Chinese women with increased cardiovascular disease risk. This effect could be due to the improved serum nutrients status in the MMS group. To our best knowledge, this is the first report, in obese women with increased cardiovascular disease risk, that evaluated the effects of supplementation with multivitamin and mineral on blood pressure and CRP.

Vitamins and minerals play a key role on maintaining the levels of enzyme in many pathways of energy metabolism. They influence the expression of genes for enzymes through acting as a second messenger, enzymatic prosthetic group or the activator of transcription factor.[33-36] Furthermore, many minerals and vitamins, such as calcium, iron, zinc, magnesium, thiamin, vitamin D, etc. play important roles not only in the process of energy metabolism but also in the regulation of blood pressure.[19-22] It is well recognized that the energy intake of obese individuals are higher than normal weight individuals. Previous studies have shown that obese individuals are more likely to have either lower blood concentrations or lower bioavailability of minerals and/or vitamins to some degrees.[15-18] Taken together, obese individuals may need to use higher amount of vitamins and minerals for the higher burden of energy, which could lead to less vitamins and minerals available for the regulation of blood pressure.

Some studies have shown that dietary calcium could reduce blood pressure, and the mechanism has been suggested to be the suppression of 1, 25-dihydroxyvitamin D, which could increase vascular smooth muscle intracellular calcium, and thus increase peripheral vascular resistance and blood pressure.[37-39] The purpose of calcium group in this study is to evaluate whether vitamins and minerals besides calcium play important roles in regulating blood pressure in obese women. In the present study, after 26-week supplementation of multivitamin and mineral or calcium, although there was a non-significant trend of lower DBP in both MMS and calcium groups compared to baseline, only MMS group had significantly lower SBP and DBP in comparison to the placebo group at trial-end. These results suggest that vitamins and minerals acted in conjunction, and hence a combination of these micronutrients had greater effect in reducing blood pressure than supplementation with calcium alone. Consistent with our results, a study on the effect of vitamins C and E, magnesium and zinc supplementation on blood pressure in type 2 diabetes patients[40] showed that the supplementation of all four elements, but not each individual vitamin or mineral, for at least three months could reduce systolic, diastolic and mean blood pressure significantly. Both the results of the study mentioned above [40] and the results of our study suggest that the combination of multivitamin and mineral could reduce the blood pressure more effectively than the supplementation of a single vitamin or mineral.

Previous studies in general population on the effects of one or several kinds of antioxidants and/or minerals supplementation have shown conflicting results.[8-14] Mid- to long-term clinical trials (7 months – 7 years) failed to show any beneficial effects on reducing the risk of hypertension,[10,13,14] and short-term trials (1–6 months) have yielded inconsistent results. Whereas some studies showed that blood pressure decreased in the supplemented group,[8-9] the others showed no change [11,12] or increased blood pressure.[41] In contrast, with multivitamin and mineral supplementation, both a long-term 6-year study with 3,318 men and women[42] and the short-term study reported here have shown significant effects on reducing blood pressure. In addition, the reduction in both diastolic and systolic pressure observed in the present study was greater than the reduction observed in the general population.[42] This may be due to the differences in the population studied, the study design or the source of multivitamin and mineral used. Obese people are of higher risk of low blood concentration of minerals and/or vitamins than the general population, which may partly explain the better effects of multivitamin and mineral supplementation on blood pressure observed in the present study with obese as the subjects. These findings further support that the effect of supplementation with multivitamin and mineral on reducing blood pressure maybe greater than supplementation with one or several kinds of minerals and/or vitamins, especially in obese individuals.

The specific biologic mechanisms for the beneficial effects of multivitamin and vitamin on blood pressure have not been fully established. The beneficial effects may be partially attributed to the actions of these micronutrients in conjunction. Some studies have suggested that vitamin B1,[22] vitamin B6,[43] vitamin C,[44] vitamin D,[19] calcium,[45] magnesium,[46] zinc,[20] play important roles in improving hypertension through modulating vascular tone, membrane ion exchange, vascular smooth muscle function, activation of Na^+ K^+-ATPase and the restoration of NO-mediated flow-dependent vasodilation.

Obesity with its related metabolic disorders is associated with chronic low-grade inflammation. Systemic inflammation is associated with reduced serum concentrations of vitamins A,[47] B-6,[48] and C [49] and inflammation may involve in the initiation as well as the development of hypertension.[50] C-reactive protein has been shown to be not only an inflammatory marker but also a direct cause of hypertension.[28,50] In a post hoc subgroup analysis of a randomized, double-blind, placebo-controlled trial, the use of a commercially available multivitamin and mineral (24-ingredient) was found to be able to reduce C-reactive protein levels.[51] In the present study, the C-reactive protein level was reduced in the MMS group, which suggests that inflammation was alleviated after 26-week multivitamin and mineral supplementation in full dose.

With regard to the supplementation amount, our study showed that the group which received one tablet of high-dose multivitamin and mineral daily, but not the group which received the low-dose tablet daily, had significantly higher serum concentrations of calcium, zinc, thiamin and riboflavin at trial-end compared to that of baseline and the placebo group. Consistently, the effects on

blood pressure were only seen in the group which received high-dose tablet daily, suggesting that adequate amount of vitamins and minerals are needed for the control of blood pressure.

In conclusion, our study results showed that, in obese Chinese women with increased cardiovascular risk factors, supplementation with a commercially available 29-ingredient multivitamin and mineral supplement can reduce blood pressure and serum level of C-reactive protein. Intake of multivitamin and mineral at sufficient amount may be a novel regimen for hypertension prevention and therapy in obese individuals.

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AUTHOR DISCLOSURES

None of the authors have any conflicts of interest.

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Original Article

Effects of supplementation with multivitamin and mineral on blood pressure and C-reactive protein in obese Chinese women with increased cardiovascular disease risk

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補充多种礦物质与维生素对中国有心血管疾病風險的肥胖婦女血压和 C 反应蛋白的影响

目的：以高血压、高血脂或高血糖的心血管疾病风险增加的肥胖女性为研究对象，评价补充多种矿物质与维生素对血压和 C 反应蛋白(CRP)的影响。对象和方法：选择 128 名年龄在 18-55 岁且心血管疾病风险高的肥胖中国女性，进行 26 周的随机、双盲、安慰剂对照试验。研究对象被随机分成 4 组，分别是每天服用一錠高剂量多种维生素与矿物质(MMS)组、一錠低剂量多种维生素与矿物质(Low MMS)组、补钙组(162mg)和安慰剂组。检测了基线和实验后的舒张压(DBP)、收缩压(SBP)和血清中的 CRP 水平。结果：在基线水平，研究对象平均年龄 42.0±7.1 岁，平均 BMI 为 30.9±2.8 kg/m^2，且四组间各种指标没有显著不同。117 个研究对象完成了试验，补充 26 周后，MMS 组同对照组相比，DBP 和 SBP 显著降低($p < 0.05$)。MMS 组和补钙组干预后同基线相比 DBP 有不显著的降低趋势($p < 0.08$)。在第 26 周，MMS 组与基线及安慰剂组相比，血清的 CRP 水平显著降低($p < 0.05$)。结论：我们的研究表明，对于心血管疾病风险增加的肥胖女性，充分补充多种维生素和矿物质可以降低血压和血清中的 CRP 水平。

關鍵字：肥胖、血压、C 反应蛋白、多种礦物质与维生素補充、中国婦女