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Inverse association between dietary vitamin C and retinol intake and hyperhomocysteinemia prevalence in middle-aged and older adults with hypertension: a cross-sectional study

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Abstract

Objectives: Plasma total homocysteine (tHcy) has been implicated in the development of cardiovascular disease. This study aimed to assess the relationship of dietary antioxidant vitamins intake with hyperhomocysteinemia (HHcy) prevalence in middle-aged and older adults with hypertension.

Design: A cross-sectional study.

Setting: The survey was conducted in the Nanshan district of Shenzhen.

Participants: A total of 1465 middle-aged and older adults with hypertension were included between July and September of 2013.

Measurements: Some dietary antioxidant vitamins (vitamin C (VC) and vitamin E (VE), carotenes, retinol, lutein) intake was estimated using the food frequency questionnaire. Socio-demographic and potential covariates were evaluated through questionnaires, anthropometric measurements and blood tests. Multiple logistic regression models were used to determine odds ratios (ORs) and 95% confidence intervals (CIs).

Results: Compared with the lowest quartile in the fully adjusted model, the ORs (95% CIs) for HHcy level across quartiles of dietary VC intake were 0.87 (0.60, 1.26), 0.53 (0.34, 0.82) and 0.43 (0.23, 0.82) ($P_{for\ trend}$=0.005), and the ORs (95% CIs) in the third quartile of retinol intake was 0.50 (0.33, 0.76), while the effect for the highest quartile was not significant ($P_{for\ trend}$=0.488). No significant association was observed between dietary VE, carotenes and lutein intake and HHcy.

Conclusions: A linear inverse association between dietary VC intake and HHcy prevalence, and a U-shaped association between dietary retinol intake and HHcy prevalence were found in Chinese middle-aged and older adults with hypertension.

Keywords: Hyperhomocysteinemia, Hypertension, Antioxidant vitamin, Vitamin C, Retinol

Strengths and limitations of this study:

This study focused on the risk of hyperhomocysteinemia among middle-aged and

older adults with hypertension.

A cross-sectional study could not explain the temporal relationship between dietary antioxidant intake and tHcy.

Although some confounding factors, such as a sedentary lifestyle and a history of disease and medicine use were included in the analysis, other potential confounders may exist.

Introduction

Increasing evidence has shown that elevated total homocysteine (tHcy) levels are associated with atherosclerotic cardiovascular diseases, stroke, peripheral arterial occlusive disease, and venous thrombosis (1, 2). Hyperhomocysteinemia (HHcy) (tHcy $\geq$ 15 μmol/L) (3), the result of a disturbed methionine metabolism, may lead to an enhancement of the adverse effects of risk factors like hypertension on human health (4). Hypertension is a major risk factor for cardiovascular and chronic kidney disease and a major cause of the global burden of disease and mortality (5, 6). It was estimated that the prevalence of hypertension in China in 2015 was 23.2%, and about 245 million Chinese adults had hypertension (7). Notably, the incidence of hypertension with hyperhomocysteinemia, or 'H-type hypertension', is significantly higher compared with other countries, representing 75% of Chinese patients with hypertension (8). Thus, the nutritional treatment focus on the attempt to reduce cardiovascular risk by reducing tHcy is important, particularly among patients with hypertension (9).

Increasing age, male sex, genetic factors, consumption of alcohol or tobacco, kidney function and physical activity are some of the factors associated with tHcy levels (10). Epidemiological studies and clinical trials have indicated that folate, vitamin B_{12} and vitamin B_6 status, well-known predictors of tHcy, are important for tHcy metabolism. The latest meta-analysis demonstrated that a lower risk of stroke and overall cardiovascular disease (CVD) with folic acid supplementation, which may partly contribute to the decrease of tHcy level (11). Folate, a key factor of tHcy metabolism, is very sensitive to free radicals (12). There are many studies have shown

the antioxidant vitamins may influence the tHcy levels for the protective effect of folate from oxidation. Plasma levels or dietary intake of vitamin C (VC), vitamin E (VE) or β-carotene was inversely associated with tHcy levels, however, the findings were not consistent (13-15). Of note, the association has never been investigated among the hypertensive population.

Therefore, this large population-based study aimed to determine the association between the dietary intake of VC, VE, carotenes, retinol and lutein and the prevalence of HHcy in middle-aged and older men and women with hypertension.

Methods

Study design and population

This study consecutively recruited individuals with hypertension from 60 community health service centres (CHSCs) in the Nanshan district of Shenzhen from July to September of 2013 using a three-stage random sampling method. In the first stage, 8 sub-districts were selected in the Nanshan district; in the second stage, 6 to 8 communities were selected from each sub-district using a simple random selection procedure; and in the third stage, individuals with hypertension were selected from each community using isometric random sampling. All subjects were of Chinese ethnicity and had lived in the Nanshan district of Shenzhen for over six months. The individuals were invited to visit the CHSCs, where the researcher-administered questionnaire (including the validated food frequency questionnaire (FFQ)) was conducted, the anthropometric measurements were recorded, and fasting blood samples were collected. The survey protocol was approved by the Ethics Committee of the Shenzhen Nanshan Centre for Chronic Disease Control, and all participants provided written informed consent before enrolment.

We collected the data of 1802 participants, and excluded 51 participants whose age was not reported or who were aged ≤ 40 years and 27 underweight participants (body mass index (BMI) < 18.5 kg/m^2), as well as participants for whom fasting blood samples (n = 74) and complete dietary surveys (n = 133) were not available. We also excluded participants with an extreme dietary energy intake (male: < 800 kcal or > 4000 kcal, female: < 700 kcal or > 3500 kcal) (n = 52). Finally, 1465 participants

were included in the analysis **(Supplementary Figure.1)**.

Patient and public involvement

The development of standardised form is in response to the public health need of preventing stroke among hypertension population. Patients and the public were not involved in the design of the study. The results of our study will be disseminated through open access publications.

Dietary assessment

The researcher-administered FFQ consisted of 92 food items, which were assembled into 7 food groups: cereals, vegetables, fruits, beans, animal foods, nuts and beverages. The FFQ was based on the national FFQ used in the 2010-2012 China National Nutrition and Health Survey according to the Chinese Nutrition and Health Surveillance in 2010-2012 (16). Participants were asked to recall the consumption of each item during the past year, including the type of food, frequency and amount. Food weight maps were available for participants to estimate their portion size. Primary data obtained from FFQs were doubly entered into the EpiData 3.0 software by two trained technicians to verify accuracy. Dietary energy and other nutrients were calculated based on the Chinese Food Composition Database (17, 18). Dietary intake of VC, VE, carotenes (consist of α-carotene, β-carotene and β-cryptoxanthin), and retinol were calculated based on the Chinese Food Composition Table 2002 (17). Dietary intake of lutein was calculated based on the food composition table of vegetables, fruits, eggs and nuts that contain large amounts of lutein (Chinese Dietary Reference Intakes 2013). The intake of all dietary nutrients and carotenoids was adjusted for energy using the residual method (19). The second FFQ was conducted 3 months after the completion of the first FFQ among 108 participants. The intra-class correlation coefficients of two administrations of FFQ for nutrients ranged from 0.044 (iodine) to 0.562 (phosphorus) and were all statistically significant, except for iodine.

Assessment of other covariates

Participants' socio-demographic characteristics (e.g., age and sex), lifestyle factors (e.g., sedentary time), history of chronic diseases, and medication and supplement use status were collected. Sedentary time consisted of time spent watching TV and sitting,

which were combined into one variable with 2 categories, < 3 h/d and ≥ 3 h/d, based on a median sedentary time of 3 h/d. The history of cardiovascular events including coronary heart disease, cerebral haemorrhage, cerebral thrombosis, and the history of diabetes and kidney disease were recorded for each participant. The prescription use status was classified into 2 groups (yes or no) corresponding to whether the participant was taking any type or quantity of drugs, including antihypertensive drugs, antihyperglycaemic drugs, lipid-lowering drugs or tHcy-lowering drugs. Supplement use consisted of vitamin A, retinol, B vitamins, VC, VE and folic acid was included.

Anthropometric measurements

Height and weight and waist circumference (WC) were measured by specialists. BMI was calculated as weight (kg) / height (m^2). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured from the right arm of participants in a seated position after a sufficient rest period using a mercury sphygmomanometer in the morning. Blood pressure was measured manually and recorded as the average of three measurements.

Laboratory tests and outcomes

Fasting blood samples were collected from the participants at CHSCs and transported under refrigerated conditions to a clinical laboratory of the Nanshan Centre for Chronic Disease Control on the same day. Blood samples were collected through deposition and centrifugation for ten minutes at 3000 s/min at room temperature. The concentrations of plasma tHcy, fasting plasma glucose (FPG), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), uric acid and creatinine were assessed on the day of blood collection using enzymatic methods via an auto-analyser (HITACH 7080). The inter-day quality control assessments met the standard during the analysis. HHcy was defined as plasma tHcy concentration ≥ 15 μmol/L.

Statistical analysis

Demographic characteristics were described by means ± SDs for normally distributed data, medians (interquartile ranges, IQRs) for non-normally distributed data and numbers (percentages) for categorical data. The differences between males and

females were compared using the *t* test for normally distributed variables, the Kruskal-Wallis rank test for non-normally distributed variables, and the chi-square test for categorical variables.

The associations between dietary antioxidant vitamins intake and the prevalence of HHcy were analyzed using multiple logistic regression models, with the lowest quartile as the reference category. Potential confounders related to dietary intake of antioxidant vitamins and HHcy reported in previous literature were chosen in multivariable analyses. The first model was adjusted for age, sex ('male' as the reference) and BMI. The second model was further adjusted for sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of protein, fat, cholesterol, fiber, folic acid, vitamin B_6 and vitamin B_{12}, saturated fatty acid, selenium, and the levels of TC, TG, LDL-C, FPG, uric acid and creatinine, which were .antihypertensive drug use (yes), antihyperglycaemic drug use (yes), and lipid-lowering drug use (yes). The third model was further adjusted for the history of cardiovascular events and kidney disease ('no' as the reference), antihypertensive drug use ('no' as the reference), antihyperglycaemic drug use ('no' as the reference), and lipid-lowering drug use ('no' as the reference). Linear trends were tested by creating a continuous variable for dietary antioxidant vitamins intake using the median value for each quartile. The sensitive analyses between dietary antioxidant vitamins intake and HHcy prevalence were applied among the population who had never suffered cardiovascular events, or among the population who never use the tHcy-lowering drug.

To further explore the nonlinearity of the relationship between dietary antioxidant vitamins intake and high tHcy, we used restricted cubic spline with 4 knots at the 5^{th}, 35^{th}, 65^{th} and 95^{th} percentiles of dietary antioxidant vitamins intake.

To evaluate the modification effect by some potential prevalence factors of HHcy, including sex (male or female) and BMI (<24 or ≥24 kg/m^2), stratified analyses were conducted by these potential factors and estimated *P* values for interaction terms.

All data were analyzed using the R Foundation version 3.1.2 and Empower X&Y Solutions Inc. Statistical significance was considered when $P < 0.05$ (two-sided).

Results

In this cross-sectional study, 1465 participants (male: 729, female: 736) were included in the analysis, and the mean ± SD of age and BMI was 62.0 ± 10.7 years, and 24.9 ± 3.5 kg/m^2. The tHcy level of the participants was 14.63 ± 9.06 μmol/L, and the number (percentage) of HHcy was 469 (32.0%). The participants with HHcy were older and more male, and have higher WC, SBP, tHcy, uric acid, creatinine, and higher percentages of history of cardiovascular events (including coronary heart disease, cerebral hemorrhage, cerebral thrombosis) and kidney disease, and have lower FPG level (all $P < 0.05$) (**Table 1**).

The dietary intake of nutrients were shown in **Table 2**. The median (IQR) of dietary antioxidant vitamins intake were as follows: VC 153.5 (91.2, 240.9) mg/d, VE 25.0 (19.3, 31.9) mg/d, carotenes 3.3 (1.8, 5.6) mg/d, retinol 138.3 (78.0, 283.6) μg/d, and lutein 9.5 (5.2, 14.8) mg/d. Among the participants with HHcy, the intake of carbohydrate was higher, and the intake of fruit, protein, fibre, retinol, folic acid, vitamin B_{12}, saturated fatty acid, selenium, magnesium, zinc was lower, and the percentage of supplement use was lower (all $P < 0.05$).

The association between dietary antioxidant vitamins intake and the HHcy prevalence are shown in **Table 3**. After adjusting for age, sex and BMI, the ORs (95% CIs) for HHcy prevalence across quartiles of VC intake were 1.00, 0.92 (0.66, 1.27), 0.64 (0.46, 0.90), and 0.55 (0.34, 0.89) ($P_{\text{for trend}} = 0.048$); the ORs (95% CIs) across quartiles of retinol intake were 1.00, 0.84 (0.61, 1.16), 0.50 (0.35, 0.70), and 0.55 (0.40, 0.77) ($P_{\text{for trend}} = 0.003$). In the fully adjusted model, the significant association was found in the third and highest quartile of VC intake, and the ORs (95% CIs) were 0.53 (0.34, 0.82) and 0.43 (0.23, 0.82) ($P_{\text{for trend}} = 0.005$); the significant association was only found in the third quartile of retinol intake, and the ORs (95% CIs) was 0.50 (0.33, 0.76) ($P_{\text{for trend}} = 0.488$). However, the non-significant association between dietary intake of VE, carotenes and lutein and HHcy prevalence was found. The results were similar in the sensitivity analyses that excluded participants with cardiovascular events or who use the tHcy-lowering drug (see **Supplementary Table 1**).

After fully adjusting for the potential confounders, the association between dietary antioxidant vitamins intake and the prevalence of HHcy were shown in **Figure.1**. From the cubic splines, we noted that the linear inverse trend of VC intake and HHcy prevalence (*P* for overall association=0.019, *P* for nonlinearity=0.058) and the U-shaped relationship of retinol intake and HHcy prevalence (*P* for overall association=0.010, *P* for nonlinearity=0.010), which were consistent with the results of logistic regression analyses. The non-association between carotenes, lutein and VE and HHcy was not shown.

In stratified analyses, the association between dietary VC and retinol intake and HHcy prevalence were not significantly modified by sex (male or female), BMI (<24 or ≥24 kg/m^2) (all $P_{\text{for interaction}}$ were >0.05) (**Figure.2**). Similar results of stratified analyses of carotenes, lutein and VE were not shown.

Discussion

In this community-based cross-sectional study, we observed some of the antioxidant vitamins intake were significantly correlated with the prevalence of HHcy. After adjusting potential confounders, a linear inverse association between VC intake and HHcy prevalence, and a U-shaped relationship between retinol intake and HHcy prevalence were found, which were not modified by sex or BMI. However, the non-significant effect of VE, carotenes and lutein on HHcy was detected.

Numerous studies have suggested that HHcy may be a modifiable risk factor for CVD, especially for stroke. In the past decades, there were many clinical trials aimed to show the effect of folic acid and vitamins B_{12} and B_6 supplement on lowering the level of tHcy, however, the negative results were found in most studies (20). The potential reason may be correlated with the harmful effect of unmetabolized excessive folate or the cyanide and thiocyanate from excessive cyanocobalamin. In China, where folate fortification has not been implemented, folic acid significantly reduced the risk of stroke was observed from the China Stroke Primary Prevention Trial (CSPPT) (21). Thus, appropriate B vitamins therapy is of great importance for lowering tHcy level in stroke prevention.

Notably, folate, which exists in blood and tissues mainly in a labile form, is very

sensitive to oxidative stress (12). In the past decades, several researches reported the potential association between plasma levels or intake of different antioxidant vitamins and tHcy level (13, 14, 22-24). In 1999, Brude IR *et al.* observed the inverse association between plasma tHcy concentration and dietary intake of vegetables, vitamin C and β-carotene from 41 participants (22). In addition, dietary intake of retinol equivalents, β-carotene and VC were inversely correlated with plasma tHcy level, after adjustment for dietary B-vitamins (14). What's more, the study focus on the effect of antioxidant vitamins on the plasma tHcy level in a free-living elderly population found that plasma VC, rather than the intake and supplementation of VC, showed a negative association with tHcy in simple regression analysis, and also found that the plasma levels, as well as the intake and supplementation of vitamin E, and β-carotene were not associated with tHcy (13). Similarly, the cross-sectional NHANES 1999–2002 study found that dietary VC and VE intake were associated with a lower prevalence of elevated blood tHcy concentration, whereas no association between dietary carotenes intake and tHcy was detected (23).

Consistent with our findings, these studies have a common conclusion that VC intake was inversely correlated with tHcy level. Given the report of an interaction of VC and folate (25), Magana AA *et al.* found the underlying molecular mechanisms that VC activates the folate-mediated one-carbon cycle in C2C12 myoblasts (26). Thus, VC has been explored as an attractive factor to increase circulating levels of folic acid and to reduce Hcy levels.

We found a U-shaped association between dietary retinol intake and high tHcy prevalence, which was similar to the conclusion that dietary intake of retinol equivalents was inversely correlated with plasma tHcy level (14). Retinol, a preformed vitamin A, plays an important role in vision, cellular differentiation, and proliferation, as well as the immune system regulation. In addition, there is increasing evidence indicates that retinol seems to inhibit thrombosis (27) and inflammation effects (28), which indicates retinol is emerging as a factor of interest to CVD. Brazionis L reported that plasma retinol was a novel marker for CVD mortality in Australian adults, with an inverse association between plasma retinol in the middle

tertile and 5-year CVD mortality (29). Similarly, a U-shaped relation between plasma retinol and the risk of stroke death was examined (30). However, a nested case-control study showed a significant inverse association between plasma retinol and the risk of first stroke among Chinese hypertensive adults from the CSPPT (31), which may due to relatively low baseline retinol concentrations (median: 67.5 μg/dL). Besides, the interaction of retinol and tHcy was found (31), which showed the effect was stronger among the participants whose tHcy < 10 μmol/L than whose tHcy ≥ 10 μmol/L.

In addition, the dietary source of retinol may contribute to the non-significant effect of retinol in the highest quartile in the fully adjusted model, as it is present in animal-based foods, particularly in liver and whole milk. In this study, we found the TG level was gradually increased with the increase of retinol (data not shown). High retinol intake may alter lipid metabolism by increasing TG levels, which may impact the tHcy metabolism (32).

At present, a few studies demonstrate the complicated relationship between retinol, tHcy and CVD risk, but the underlying mechanism has not yet been clarified (33). The antioxidant activity of retinol may be a plausible mechanism that links the effect of retinol on tHcy levels, as retinol is essential for the maintenance of immune function and antioxidant defence (34, 35).

However, we found no relationship between HHcy prevalence and dietary intake of VE. The aforementioned study has found significant inverse associations between plasma tHcy and dietary intake of VE, but the effect was not significant after adjusting B-vitamins intake, which was consistent with our finding (14). On the contrary, dietary VE (α-tocopherol) intake was associated with a lower risk of elevated blood tHcy concentration among US adults (23).

Epidemiological studies have reported the positive role of carotenoids on human health. In this study, we focused on the effect of carotenes (β-carotene, α-carotene and β-cryptoxanthin) and lutein on tHcy, however, the negative results were found. Many of the aforementioned studies reported the protective effect of β-carotene by lowering tHcy concentration (14, 22), but the risk of tHcy > 13 μmol/L was associated with the

total carotene intake from diet plus supplement use, rather than the only intake from diet (23). The negative finding of lutein cannot be compared because of a lack of previously reported data. Thus, more prospective cohort studies and randomized double trials are warranted to indicate the relationship of antioxidant vitamins with HHcy risk.

The strength of this study is the face-to-face researcher-administered FFQ survey. First, to our knowledge, this is the first study to demonstrate an association of dietary intake of antioxidant vitamins with HHcy prevalence that was conducted in both a male and female hypertensive population. Then, the researcher-administered face-to-face interview was considered by validated FFQ, which was designed to evaluate dietary intake and gave full consideration to eating habits and food nutrient composition in the Chinese population.

Our study has several limitations. First, the limitations of a cross-sectional study are worth considering, which prevent us from explaining the temporal relationship between dietary antioxidant intake and tHcy. Second, we investigated the participants whether they took dietary supplements, while the detailed doses were not recorded. Therefore, we are not able to eliminate the possible association between supplement use and HHcy prevalence. Nevertheless, the study reported that there was some potential benefit from the antioxidant supplementation on plasma tHcy concentration (23), which could stabilize the positive results in this study. In addition, although some confounding factors, such as a sedentary lifestyle and a history of disease and medicine use were included in the analysis, other potential confounders may exist. For instance, the influence of smoking and drinking on HHcy was not assessed because of the lack of information about the status of smoking and drinking.

In conclusion, we found dietary intake of VC and retinol was inversely associated with HHcy prevalence in middle-aged and older adults with hypertension after adjusting the potential confounders. Our findings have provided suggestive evidence of a relationship between certain antioxidant intake and HHcy, which should be the impetus for longitudinal and random control trails to verify the relationship and direction and to elucidate the underlying mechanisms in the future.

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The authors' responsibilities were as follows: H.L. conceived and designed the study and critically revised the manuscript. P.X. and G.Q. analyzed the data and wrote the paper. Z.J. participated in the laboratory assay. M.J. and Z.D. collected the data and revised the manuscript. All authors read and approved the final version of the manuscript.

All authors declare they have no conflict of interest relevant to the content of this article.

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Table 1 Basic characteristics of the participants [#]

	Total (n=1465)	HHcy (n=469)	non-HHcy (n=996)	*P*
Age (years)	62.0 ± 10.7	64.4 ± 11.4	60.9 ± 10.2	<0.001
Gender (Male, n (%))	729 (49.8)	339 (72.3)	390 (39.2)	<0.001
BMI (kg/m^2)	24.9 ± 3.5	25.0 ± 3.4	24.8 ± 3.6	0.252
WC (cm)	87.3 ± 9.5	88.7 ± 10.0	86.7 ± 9.2	<0.001
SBP (mmHg)	132.9 ± 13.5	133.9 ± 14.0	132.4 ± 13.3	0.046
DBP (mmHg)	80.4 ± 10.6	81.2 ± 11.4	80.0 ± 10.2	0.050
tHcy (μmol/L)	14.63 ± 9.06	21.57 ± 13.35	11.37 ± 1.89	<0.001
FPG (mmol/L)	5.42 ± 1.54	5.29 ± 1.35	5.49 ± 1.62	0.024
TC (mmol/L)	5.25 ± 1.64	5.20 ± 2.49	5.27 ± 1.02	0.414
TG (mmol/L)	1.95 ± 1.39	2.01 ± 1.34	1.92 ± 1.41	0.254
LDL-C (mmol/L)	3.19 ± 0.87	3.14 ± 0.89	3.21 ± 0.87	0.133
Uric acid (μmol/L)	380 ± 97	420 ± 103	362 ± 89	<0.001
Creatinine (μmol/L)	83 ± 44	98 ± 67	76 ± 25	<0.001
Sedentary time <3h/d (n (%))	667 (45.5)	211 (45.0)	456 (45.8)	0.776
History of disease (n (%))				
Coronary heart disease	178 (12.2)	72 (15.4)	106 (10.6)	0.010
Cerebral hemorrhage	15 (1.0)	11 (2.3)	4 (0.4)	<0.001
Cerebral thrombosis	61 (4.2)	34 (7.2)	27 (2.7)	<0.001
Diabetes	240 (16.4)	82 (17.5)	158 (15.9)	0.434
Kidney disease	57 (3.9)	34 (7.2)	23 (2.3)	<0.001
Medications use (n (%))				
Antihypertensive	1146 (78.2)	380 (81.0)	766 (76.9)	0.075
Antihyperglycemic	21(1.4)	8 (1.7)	13 (1.3)	0.547
Lipid-lowering	21 (1.4)	5 (1.1)	16 (1.6)	0.417

BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HHcy, hyperhomocysteinemia; LDL-C, low density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; tHcy, total homocysteine; WC, waist circumference. [#]: Values are mean ± SD or median (interquartile range) or number (percentage).

Table 2 The dietary intake of food and nutrients of the participants [#]

Dietary intake (/d)	Total (n=1465)	HHcy (n=469)	nonHHcy (n=996)	*P*
Energy (kcal)	1488 (1172, 1899)	1508 (1202-1874)	1473 (1150-1913)	0.460
Vegetable (g)	383.7 (240.3, 578.0)	377.7 (227.2, 580.0)	388.2 (252.5, 577.1)	0.510
Fruit (g)	79.2 (39.7, 139.5)	75.4 (33.2, 127.5)	81.1 (42.1, 144.6)	0.036
Protein (g)	43.2 (35.4, 53.5)	41.7 (33.9-50.9)	44.2 (36.2-54.8)	<0.001
Fat (g)	54.3 (42.2, 65.6)	52.5 (41.6-64.3)	55.1 (42.6-65.9)	0.055
Carbohydrate (g)	206.6 (179.4, 234.4)	212.6 (186.6-237.7)	204.4 (176.6-231.7)	<0.001
Cholesterol (mg)	232.7 (152.3, 325.8)	228.3 (154.5, 313.7)	236.6 (147.3, 333.6)	0.229
Fibre (g)	11.2 (8.2, 15.2)	10.6 (7.9-14.8)	11.4 (8.4-15.2)	0.021
Vitamin C (mg)	153.5 (91.2, 240.9)	138.6 (85.6-231.6)	159.5 (93.6-242.7)	0.072
Vitamin E (mg)	25.0 (19.3-31.9)	24.1 (19.0-31.5)	25.4 (19.5-32.2)	0.154
Carotenes (mg)	3.3 (1.8, 5.6)	3.1 (1.7-5.5)	3.5 (1.8-5.6)	0.228
Retinol (μg)	138.3 (78.0, 283.6)	110.9 (62.2-239.3)	152.0 (83.3-317.6)	<0.001
Lutein (mg)	9.5 (5.2, 14.8)	9.0 (5.1-14.2)	9.6 (5.3-15.0)	0.269
Folic acid (μg)	170.4 (109.4, 263.5)	160.0 (99.9-246.2)	176.1 (112.8-266.9)	0.009

continued on next page

Table 2 *(continued)*

	Total	HHcy (n=469)	non-HHcy (n=996)	*P*
Vitamin B_6 (mg)	0.3 (0.2, 0.5)	0.3 (0.2-0.5)	0.3 (0.2-0.5)	0.233
Vitamin B_{12} (μg)	2.7 (1.6, 4.6)	2.3 (1.4-4.0)	3.0 (1.8-4.9)	<0.001
Saturated fatty acid (g)	10.6 (8.1-13.2)	10.2 (7.8-12.9)	10.8 (8.3-13.3)	0.016
Monounsaturated fatty acid (g)	12.7 (9.8-16.3)	12.4 (9.7-16.1)	12.9 (9.9-16.3)	0.111
Polyunsaturated fatty acid (g)	20.0 (14.9-26.4)	19.7 (14.9-26.0)	20.2 (15.0-26.7)	0.292
Selenium (μg)	31.3 (23.2, 41.9)	28.6 (21.0-38.8)	32.5 (24.5-43.7)	<0.001
Magnesium (mg)	216.1 (173.3- 269.2)	205.8 (167.7-261.5)	221.0 (176.3-271.6)	0.011
Zinc (mg)	7.0 (6.0- 8.3)	6.8 (5.9-7.9)	7.1 (6.1-8.5)	<0.001
Iron (mg)	16.6 (12.0- 23.4)	16.5 (11.8-23.1)	16.6 (12.1-23.6)	0.295
Supplement use (n (%))	302 (20.6)	80 (17.1)	222 (22.3)	0.021

[#]: Values are median (interquartile range) or number (percentage). HHcy, hyperhomocysteinemia.

Table 3 ORs (95% CIs) for hyperhomocysteinemia prevalence according to quartiles (Q) of energy-adjusted antioxidant vitamins intake among the middle-aged and older hypertensive participants

	Q1	Q2	Q3	Q4	$P_{\text{for trend}}$
Vitamin C (mg/d)	< 91.2	91.2-153.5	153.6-240.9	≥ 240.9	
Cases (%)	132 (36.1)	122 (33.3)	104 (28.4)	111 (30.2)	
Age-, sex- and BMI- adjusted #	Ref.	0.92 (0.66, 1.27)	0.64 (0.46, 0.90)	0.55 (0.34, 0.89)	0.048
Multivariable-adjusted $	Ref.	0.89 (0.62, 1.28)	0.53 (0.34, 0.81)	0.46 (0.24, 0.86)	0.007
Multivariable-adjusted *	Ref.	0.87 (0.60, 1.26)	0.53 (0.34, 0.82)	0.43 (0.23, 0.82)	0.005
Vitamin E (mg/d)	< 19.3	19.3-25.0	25.1-31.9	≥ 31.9	
Cases (%)	122 (33.6)	126 (34.2)	113 (30.9)	108 (29.3)	
Age-, sex- and BMI- adjusted #	Ref.	1.10 (0.79, 1.53)	0.96 (0.69, 1.34)	0.95 (0.68, 1.33)	0.618
Multivariable-adjusted $	Ref.	1.31 (0.92, 1.88)	1.13 (0.76, 1.69)	1.18 (0.70, 1.99)	0.670
Multivariable-adjusted *	Ref.	1.26 (0.87, 1.81)	1.05 (0.70, 1.58)	1.08 (0.64, 1.83)	0.946
Carotenes (mg/d)	< 1.78	1.78-3.30	3.31-5.61	≥ 5.61	
Cases (%)	118 (32.7)	136 (37.0)	99 (26.8)	116 (31.6)	
Age-, sex- and BMI- adjusted #	Ref.	1.22 (0.88, 1.69)	0.72 (0.51, 1.01)	0.93 (0.67, 1.30)	0.254

continued on next page

Table 3 (*continued*)

	Q1	Q2	Q3	Q4	$P_{\text{for trend}}$
Multivariable-adjusted [$]	Ref.	1.17 (0.82, 1.68)	0.68 (0.45, 1.03)	0.70 (0.39, 1.27)	0.103
Multivariable-adjusted [*]	Ref.	1.19 (0.83, 1.71)	0.71 (0.47, 1.08)	0.73 (0.40, 1.33)	0.136
Retinol (μg/d)	< 78.0	78.0-138.3	138.4-283.6	≥ 283.6	
Cases (%)	147 (40.2)	133 (36.3)	91 (24.9)	98 (26.7)	
Age-, sex- and BMI- adjusted [#]	Ref.	0.84 (0.61, 1.16)	0.50 (0.35, 0.70)	0.55 (0.40, 0.77)	0.003
Multivariable-adjusted [$]	Ref.	0.80 (0.56, 1.14)	0.49 (0.32, 0.74)	0.65 (0.41, 1.05)	0.536
Multivariable-adjusted [*]	Ref.	0.81 (0.56, 1.15)	0.50 (0.33, 0.76)	0.65 (0.40, 1.05)	0.488
Lutein (mg/d)	< 5.22	5.22-9.48	9.49-14.82	≥ 14.82	
Cases (%)	123 (33.7)	120 (32.8)	114 (31.1)	112 (30.4)	
Age-, sex- and BMI- adjusted [#]	Ref.	0.98 (0.71, 1.37)	0.84 (0.61, 1.17)	0.87 (0.62, 1.22)	0.331
Multivariable-adjusted [$]	Ref.	1.05 (0.73, 1.51)	0.88 (0.58, 1.34)	0.82 (0.44, 1.51)	0.438
Multivariable-adjusted [*]	Ref.	1.01 (0.70, 1.46)	0.88 (0.58, 1.34)	0.78 (0.42, 1.46)	0.392

BMI, body mass index; FPG, fasting plasma glucose; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride

[#]: Adjusted for age, sex ('male' as the reference), BMI.

[$]: Adjusted for variables in Model # and further adjusted for sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of protein, fat, cholesterol, fiber, folic acid, vitamin B_6 and vitamin B_{12}, saturated fatty acid, selenium, and the levels of TC, TG, LDL-C, FPG, uric acid and creatinine.

*: Adjusted for variables in Model $ and further adjusted for the history of cardiovascular events and kidney disease('no' as the reference), antihypertensive drug use ('no' as the reference), antihyperglycaemic drug use ('no' as the reference), and lipid-lowering drug use ('no' as the reference).

Figure Legends:

Fig. 1 Restricted cubic spline analyses illustrating the shapes of multivariable association between dietary vitamin C intake (A), or dietary retinol intake (B) and HHcy. Adjusted for age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of protein, fat, cholesterol, fiber, folic acid, vitamin B_6 and vitamin B_{12}, saturated fatty acid, selenium, and the levels of TC, TG, LDL-C, FPG, uric acid and creatinine, the history of cardiovascular events and kidney disease ('no' as the reference), antihypertensive drug use ('no' as the reference), antihyperglycaemic drug use ('no' as the reference), and lipid-lowering drug use ('no' as the reference).

Fig. 2 ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to vitamin C, retinol intake in subgroups, stratified by sex (A), BMI (B). Adjusted for age, sex (except sex subgroup), BMI (except BMI subgroup), sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of protein, fat, cholesterol, fiber, folic acid, vitamin B6 and vitamin B12, saturated fatty acid, selenium, and the levels of TC, TG, LDL-C, FPG, uric acid and creatinine, the history of cardiovascular events and kidney disease ('no' as the reference), antihypertensive drug use ('no' as the reference), antihyperglycaemic drug use ('no' as the reference), and lipid-lowering drug use ('no' as the reference).

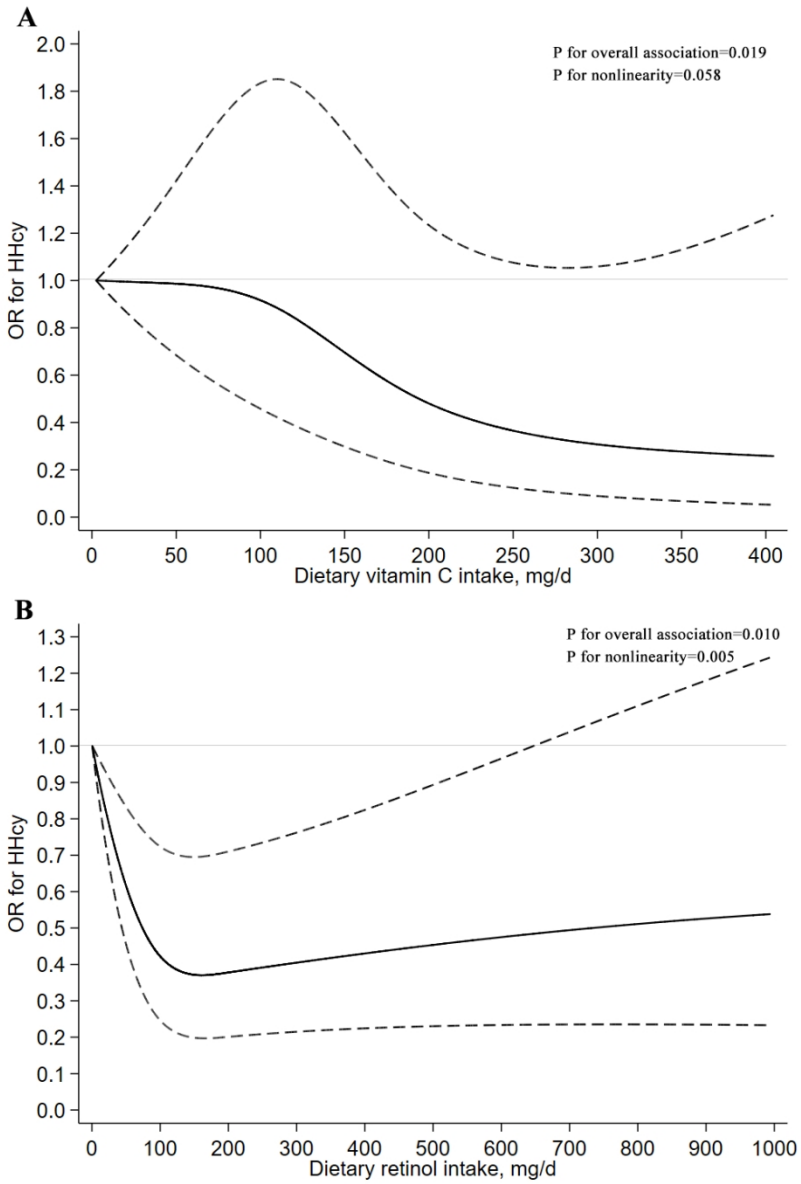

Figure 1

Restricted cubic spline analyses illustrating the shapes of multivariable association between dietary vitamin C intake (A), or dietary retinol intake (B) and HHcy. Adjusted for age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of protein, fat, cholesterol, fiber, folic acid, vitamin B6 and vitamin B12, saturated fatty acid, selenium, and the levels of TC, TG, LDL-C, FPG, uric acid and creatinine, the history of cardiovascular events and kidney disease ('no' as the reference), antihypertensive drug use ('no' as the reference), antihyperglycaemic drug use ('no' as the reference), and lipid-lowering drug use ('no' as the reference).

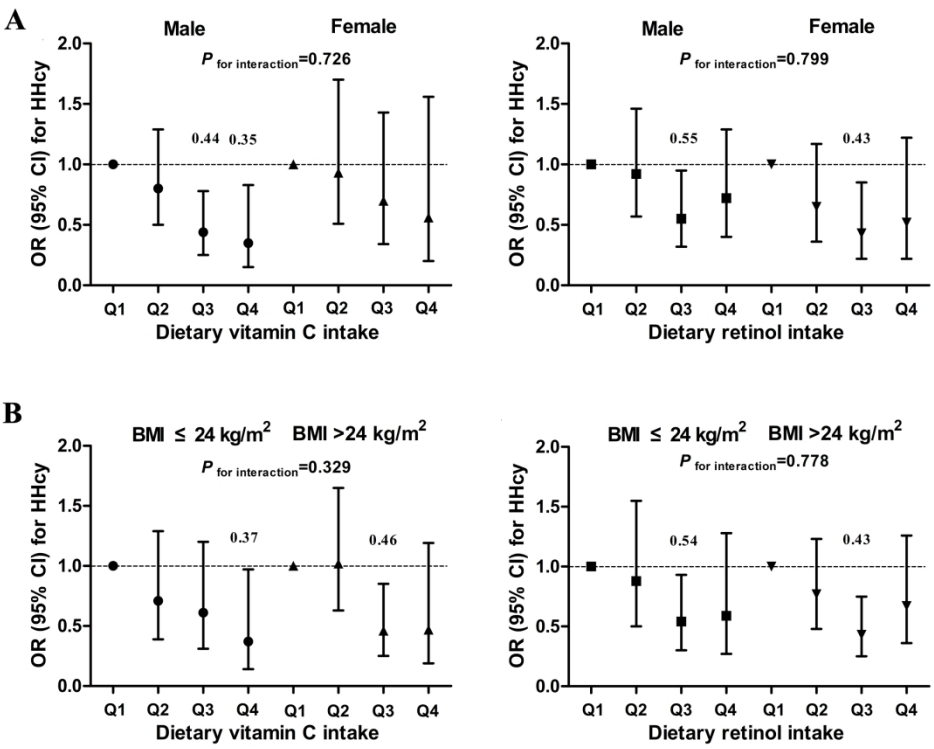

Figure 2
ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to vitamin C, retinol intake in subgroups, stratified by sex (A), BMI (B). Adjusted for age, sex (except sex subgroup), BMI (except BMI subgroup), sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of protein, fat, cholesterol, fiber, folic acid, vitamin B6 and vitamin B12, saturated fatty acid, selenium, and the levels of TC, TG, LDL-C, FPG, uric acid and creatinine, the history of cardiovascular events and kidney disease ('no' as the reference), antihypertensive drug use ('no' as the reference), antihyperglycaemic drug use ('no' as the reference), and lipid-lowering drug use ('no' as the reference).

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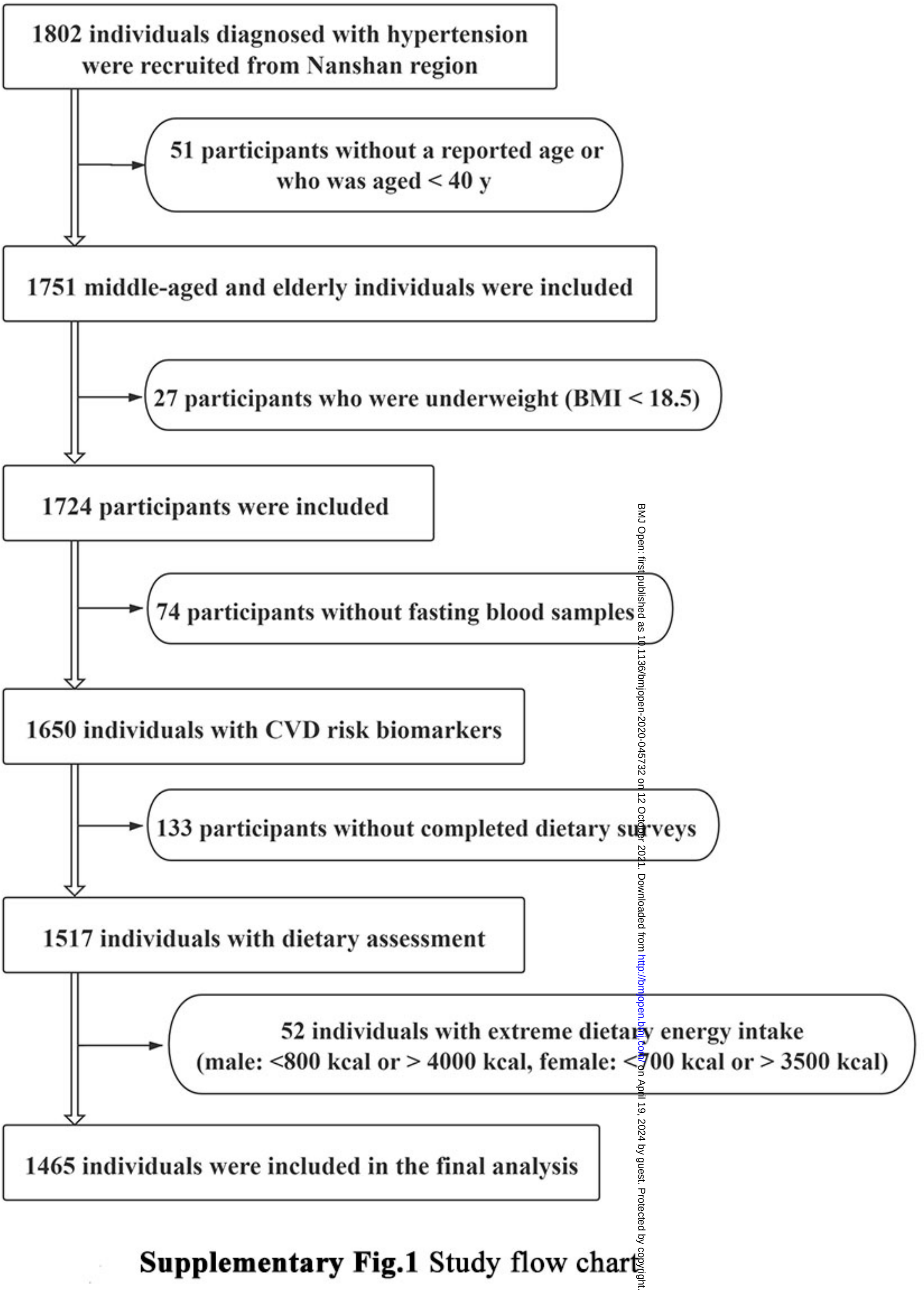

Supplementary Fig.1 Study flow chart

Supplementary Table 1 Sensitivity analyses of ORs (95% CIs) of antioxidant vitamins intake for hyperhomocysteinemia prevalence in multivariable-adjusted model among the patients with hypertension [#]

	Q1	Q2	Q3	Q4	$P_{\text{for trend}}$
Without cardiovascular events (n=1245)					
Vitamin C	1.00 (Ref.)	0.93 (0.62, 1.40)	0.55 (0.34, 0.89)	0.40 (0.19, 0.82)	0.005
Vitamin E	1.00 (Ref.)	1.16 (0.78, 1.72)	0.97 (0.62, 1.52)	0.93 (0.53, 1.64)	0.668
Carotenes	1.00 (Ref.)	1.23 (0.82, 1.85)	0.69 (0.43, 1.09)	0.69 (0.36, 1.34)	0.115
Retinol	1.00 (Ref.)	0.95 (0.64, 1.42)	0.58 (0.37, 0.92)	0.72 (0.43, 1.23)	0.518
Lutein	1.00 (Ref.)	1.08 (0.72, 1.62)	0.83 (0.52, 1.31)	0.68 (0.34, 1.36)	0.208
Without tHcy-lowering drug-using (n=1360)					
Vitamin C	1.00 (Ref.)	0.92 (0.61, 1.37)	0.54 (0.33, 0.88)	0.50 (0.24, 0.97)	0.032
Vitamin E	1.00 (Ref.)	1.25 (0.83, 1.87)	1.13 (0.72, 1.78)	1.23 (0.68, 2.21)	0.585
Carotenes	1.00 (Ref.)	1.24 (0.83, 1.85)	0.74 (0.46, 1.19)	0.67 (0.34, 1.34)	0.122
Retinol	1.00 (Ref.)	0.79 (0.53, 1.18)	0.50 (0.32, 0.80)	0.67 (0.40, 1.13)	0.614
Lutein	1.00 (Ref.)	0.95 (0.63, 1.42)	0.86 (0.53, 1.37)	0.71 (0.35, 1.45)	0.338

BMI, body mass index; FPG, fasting plasma glucose; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride

[#]: Adjusted for age, sex, BMI, sedentary time (‘< 3 h/d’ as the reference), supplement use (‘no’ as the reference), dietary intake of protein, fat, cholesterol, fiber, folic acid, vitamin B_6 and vitamin B_{12}, saturated fatty acid, selenium, and the levels of TC, TG, LDL-C, FPG, uric acid and creatinine, and the history of kidney disease, antihypertensive drug use (‘no’ as the reference), antihyperglycaemic drug use (‘no’ as the reference), lipid-lowering drug use (‘no’ as the reference).

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	6-7
		(*c*) Explain how missing data were addressed	6-7
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	6-7
		(*e*) Describe any sensitivity analyses	6-7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	4
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	7
Outcome data	15*	Report numbers of outcome events or summary measures	7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association between dietary antioxidant vitamins intake and homocysteine levels in middle-aged and older adults with hypertension: a cross-sectional study

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Association between dietary antioxidant vitamins intake and homocysteine levels in middle-aged and older adults with hypertension: a cross-sectional study

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Abstract

Objectives: Plasma total homocysteine (tHcy) has been implicated in the development of cardiovascular disease. This study aimed to assess the relationship of dietary antioxidant vitamins intake with tHcy levels in middle-aged and older adults with hypertension.

Design: A cross-sectional study.

Setting: The survey was conducted in the Nanshan district of Shenzhen.

Participants: A total of 1465 middle-aged and older adults with hypertension were included between July and September of 2013.

Measurements: Some dietary antioxidant vitamins (vitamin C (VC) and vitamin E (VE), carotenes, retinol, lutein) intake was estimated using the food frequency questionnaire. Socio-demographic and potential covariates were evaluated through questionnaires, anthropometric measurements and blood tests. The association between dietary intakes of antioxidant vitamins and tHcy concentration were evaluated by multiple linear regression analyses after ln-transformed. Multiple logistic regression models were further used to determine odds ratios (ORs) and 95% confidence intervals (CIs).

Results: The β (95% CIs) of VC intake and tHcy was -0.050 (-0.084, -0.016). Compared with the lowest quartile in the fully adjusted model, the ORs (95% CIs) for HHcy levels across quartiles of dietary VC intake were 0.82 (0.57, 1.16), 0.49 (0.33, 0.74) and 0.40 (0.22, 0.74) (*P* for trend=0.001). The β (95% CIs) of retinol intake and tHcy was -0.021 (-0.041, -0.002), and the ORs (95% CIs) in the third quartile of retinol intake was 0.61 (0.42, 0.86), while the effect for the highest quartile was not significant (*P* for trend=0.951). No significant association was observed between dietary VE, carotenes and lutein intake and HHcy.

Conclusions: A linear inverse association between dietary VC intake and HHcy prevalence, and an L-shaped association between dietary retinol intake and HHcy prevalence were found in Chinese middle-aged and older adults with hypertension.

Strengths and limitations of this study:

1. This study focused on the risk of hyperhomocysteinemia among middle-aged and

older adults with hypertension.

2. The threshold effect of retinol on HHcy was reported in this study.

3. Based on a cross-sectional study design, we could only draw a conclusion about correlation, not causation.

4. Although some confounding factors were included in the analysis, other potential confounders may exist.

Introduction

Increasing evidence has shown that elevated total homocysteine (tHcy) levels are associated with atherosclerotic cardiovascular diseases, stroke, peripheral arterial occlusive disease, and venous thrombosis (1, 2). Hyperhomocysteinemia (HHcy) (tHcy $\geq$ 15 μmol/L) (3), the result of a disturbed methionine metabolism, may lead to an enhancement of the adverse effects of risk factors like hypertension on human health (4). Hypertension is a major risk factor for cardiovascular and chronic kidney disease and a major cause of the global burden of disease and mortality (5, 6). It was estimated that the prevalence of hypertension in China in 2015 was 23.2%, and about 245 million Chinese adults had hypertension (7). Notably, the incidence of hypertension with hyperhomocysteinemia, or 'H-type hypertension', is significantly higher compared with other countries, representing 75% of Chinese patients with hypertension (8). Thus, the nutritional treatment focus on the attempt to reduce cardiovascular risk by reducing tHcy is important, particularly among patients with hypertension (9).

Increasing age, male sex, genetic factors, consumption of alcohol or tobacco, kidney function and physical activity are some of the factors associated with tHcy levels (10). Epidemiological studies and clinical trials have indicated that folate, vitamin B_{12} and vitamin B_6 status, well-known predictors of tHcy, are important for tHcy metabolism. The latest meta-analysis demonstrated that a lower risk of stroke and overall cardiovascular disease (CVD) with folic acid supplementation, which may partly contribute to the decrease of tHcy levels (11). Folate, a key factor of tHcy metabolism, is very sensitive to free radicals (12-14). There are many studies have shown the antioxidant vitamins may influence the tHcy levels for the protective effect of folate

from oxidation. Plasma levels or dietary intake of vitamin C (VC), vitamin E (VE) or β-carotene was inversely associated with tHcy levels, however, the findings were not consistent (15-17). Of note, the association has never been investigated among the hypertensive population.

Therefore, this large population-based study aimed to determine the association between the dietary intake of VC, VE, carotenes, retinol and lutein and the prevalence of HHcy in middle-aged and older men and women with hypertension.

Methods

Study design and population

This study consecutively recruited individuals with hypertension from 60 community health service centres in the Nanshan district of Shenzhen from July to September of 2013 using a three-stage random sampling method. In the first stage, 8 sub-districts were selected in the Nanshan district; in the second stage, 6 to 8 communities were selected from each sub-district using a simple random selection procedure; and in the third stage, individuals with hypertension were selected from each community using isometric random sampling. All subjects were of Chinese ethnicity and had lived in the Nanshan district of Shenzhen for over six months. The individuals were invited to visit the CHSCs, where the researcher-administered questionnaire (including the validated food frequency questionnaire (FFQ)) was conducted, the anthropometric measurements were recorded, and fasting blood samples were collected.

We collected the data of 1802 participants, and excluded 51 participants whose age was not reported or who were aged ≤ 40 years and 27 underweight participants (body mass index (BMI) < 18.5 kg/m^2), as well as participants for whom fasting blood samples (n = 74) and complete dietary surveys (n = 133) were not available. We also excluded participants with an extreme dietary energy intake (male: < 800 kcal or > 4000 kcal, female: < 700 kcal or > 3500 kcal) (n = 52). Finally, 1465 participants were included in the analysis **(Supplementary Figure 1)**.

Patient and public involvement

The development of standardised form is in response to the public health need of preventing stroke among hypertension population. Patients and the public were not

involved in the design of the study. The results of our study will be disseminated through open access publications.

Dietary assessment

The researcher-administered FFQ consisted of 92 food items, which were assembled into 7 food groups: cereals, vegetables, fruits, beans, animal foods, nuts and beverages. The FFQ was based on the national FFQ used in the 2010-2012 China National Nutrition and Health Survey according to the Chinese Nutrition and Health Surveillance in 2010-2012 (18). Participants were asked to recall the consumption of each item during the past year, including the type of food, frequency and amount. Food weight maps were available for participants to estimate their portion size. Primary data obtained from FFQs were doubly entered into the EpiData 3.0 software by two trained technicians to verify accuracy. Dietary energy and other nutrients were calculated based on the Chinese Food Composition Database (19, 20). Dietary intake of VC, VE, carotenes (consist of α-carotene, β-carotene and β-cryptoxanthin), and retinol were calculated based on the Chinese Food Composition Table 2002 (17). Dietary intake of lutein was calculated based on the food composition table of vegetables, fruits, eggs and nuts that contain large amounts of lutein, according to Chinese Dietary Reference Intakes 2013 (21). The intake of all dietary nutrients and carotenoids was adjusted for energy using the residual method (22). The second FFQ was conducted 3 months after the completion of the first FFQ among 108 participants. The intra-class correlation coefficients of two administrations of FFQ for nutrients of VC, VE, carotenes, retinol, lutein were 0.395, 0.477, 0.355, 0.551 and 0.350, and were all statistically significant.

Assessment of other covariates

Participants' socio-demographic characteristics (e.g., age and sex), lifestyle factors (e.g., sedentary time), history of chronic diseases, and medication and supplement use status were collected. Sedentary time consisted of time spent watching TV and sitting, which were combined into one variable with 2 categories, < 3 h/d and ≥ 3 h/d, based on a median sedentary time of 3 h/d. The history of cardiovascular events including coronary heart disease, cerebral haemorrhage, cerebral thrombosis, and the history of diabetes and kidney disease were recorded for each participant. The prescription use

status was classified into 2 groups (yes or no) corresponding to whether the participant was taking any type or quantity of drugs, including antihypertensive drugs, antihyperglycaemic drugs, lipid-lowering drugs or tHcy-lowering drugs. Supplement use consisted of vitamin A, retinol, B vitamins, VC, VE and folic acid was included.

Anthropometric measurements

Height and weight and waist circumference (WC) were measured by specialists. BMI was calculated as weight (kg) / height (m^2). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured from the right arm of participants in a seated position after a sufficient rest period using a mercury sphygmomanometer in the morning. Blood pressure was measured manually and recorded as the average of three measurements.

Laboratory tests and outcomes

Fasting blood samples were collected from the participants at the community health service centres and transported under refrigerated conditions to a clinical laboratory of the Nanshan Centre for Chronic Disease Control within 2 hours. The blood specimens were collected in a 5-ml EDTA vacuum tube. Blood samples were collected through deposition and centrifugation for ten minutes at 3000 r/min at room temperature. The concentrations of plasma tHcy, fasting plasma glucose (FPG), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), uric acid and creatinine were assessed on the day of blood collection using enzymatic methods via an auto-analyser (HITACHI 7080). The inter-day quality control assessments met the standard during the analysis. HHcy was defined as plasma tHcy concentration ≥ 15 μmol/L.

Statistical analysis

Demographic characteristics were described by means ± SDs for normally distributed data, medians (interquartile ranges, IQRs) for non-normally distributed data and numbers (percentages) for categorical data. The differences between males and females were compared using the *t* test for normally distributed variables, the Kruskal-Wallis rank test for non-normally distributed variables, and the chi-square test for categorical variables.

Both dietary intakes of antioxidant vitamins and tHcy concentration were ln-

transformed to improve normality before analyses and categorised into quartiles. The associations between dietary antioxidant vitamins intake and the prevalence of HHcy were analyzed using multiple logistic regression models, with the lowest quartile as the reference category. Confounding variables were selected based on the minimal sufficient adjustment recommended by the Directed Acyclic Graph, created in the online software Dagitty 3.0 (**Supplementary Figure 2**). The selected potential confounders included age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as the reference), the intakes of folate, vitamin B_6 and vitamin B_{12}, supplement use ('no' as the reference) and the history of cardiovascular events ('no' as the reference). Linear trends were tested by creating a continuous variable for dietary antioxidant vitamins intake using the median value for each quartile. The sensitive analyses between dietary antioxidant vitamins intake and HHcy prevalence were applied among the population who had never suffered cardiovascular events, or among the population who never use the tHcy-lowering drug.

To further explore the nonlinearity of the relationship between dietary antioxidant vitamins intake and high tHcy, we used restricted cubic spline with 4 knots at the 5^{th}, 35^{th}, 65^{th} and 95^{th} percentiles of dietary antioxidant vitamins intake.

To evaluate the modification effect by some potential prevalence factors of HHcy, including sex (male or female) and BMI (<24 or ≥24 kg/m^2), stratified analyses were conducted by these potential factors and estimated *P* values for interaction terms.

All data were analyzed using the R Foundation version 3.1.2 and Empower X&Y Solutions Inc. Statistical significance was considered when $P < 0.05$ (two-sided).

Results

In this cross-sectional study, 1465 participants (male: 729, female: 736) were included in the analysis, and the mean ± SD of age and BMI was 62.0 ± 10.7 years, and 24.9 ± 3.5 kg/m^2. The tHcy levels of the participants was 14.63 ± 9.06 μmol/L, and the number (percentage) of HHcy was 469 (32.0%). The participants with HHcy were older and more male, and have higher WC, SBP, tHcy, uric acid, creatinine, and higher percentages of history of cardiovascular events (including coronary heart disease, cerebral hemorrhage, cerebral thrombosis) and kidney disease, and have lower FPG

level (all $P < 0.05$) (**Table 1**).

The dietary intakes of nutrients were shown in **Table 2**. The median (IQR) of dietary antioxidant vitamins intake were as follows: VC 153.5 (91.2, 240.9) mg/d, VE 25.0 (19.3, 31.9) mg/d, carotenes 3.3 (1.8, 5.6) mg/d, retinol 138.3 (78.0, 283.6) μg/d, and lutein 9.5 (5.2, 14.8) mg/d. Among the participants with HHcy, the intake of carbohydrate was higher, and the intake of fruit, protein, fibre, retinol, folate, vitamin B_{12}, saturated fatty acid, selenium, magnesium, zinc was lower, and the percentage of supplement use was lower (all $P < 0.05$).

The association between dietary antioxidant vitamins intake and tHcy levels are shown in **Table 3**. The inverse association between VC intake and tHcy concentration after ln-transformed in the fully adjusted model, and the β (95% CIs) was -0.050 (-0.084, -0.016), which was consistent with the logistic regression as categorical variables. In Model II, the significant association was found in the third and highest quartile of VC intake, and the ORs (95% CIs) were 0.49 (0.33, 0.74) and 0.40 (0.22, 0.74) ($P_{\text{for trend}} = 0.001$). The retinol intake was also inversely associated with tHcy, as the β (95% CIs) was -0.021 (-0.041, -0.002). After adjusting for age, sex and BMI, the ORs (95% CIs) for HHcy prevalence across quartiles of retinol intake were 1.00, 0.84 (0.61, 1.16), 0.50 (0.35, 0.70), and 0.55 (0.40, 0.77) ($P_{\text{for trend}} = 0.003$). However, in Model II, the significant association was only found in the third quartile of retinol intake, and the ORs (95% CIs) was 0.61 (0.42, 0.86) ($P_{\text{for trend}} = 0.951$). The non-significant association between dietary intake of VE, carotenes and lutein and HHcy prevalence was found. The results were similar in the sensitivity analyses that excluded participants with cardiovascular events or who use the tHcy-lowering drug (see **Supplementary Table 1**).

After fully adjusting for the potential confounders, the association between dietary antioxidant vitamins intake and the prevalence of HHcy were shown in **Figure.1**. From the cubic splines, we noted that the linear inverse trend of VC intake and HHcy prevalence (*P* for overall association=0.016, *P* for nonlinearity=0.055) and the L-shaped relationship of retinol intake and HHcy prevalence (*P* for overall association=0.011, *P* for nonlinearity=0.020), which were consistent with the results of

logistic regression analyses. The threshold analysis showed that the ORs (95% CIs) of HHcy was 0.995 (0.991, 0.995) (P=0.005) when retinol intake was lower than 147.2 μg/d and 1.000 (1.000, 1.001) (P=0.094) when retinol intake was more than 147.2 μg/d, and the *P* value of log-likelihood ratio was 0.003. The non-association between carotenes, lutein and VE and HHcy was not shown.

In stratified analyses, the association between dietary VC and retinol intake and HHcy prevalence were not significantly modified by sex (male or female), BMI (<24 or ≥24 kg/m^2) (all $P_{\text{for interaction}}$ were >0.05) (**Figure 2**). Similar results of stratified analyses of carotenes, lutein and VE were not shown.

Discussion

In this community-based cross-sectional study, we observed some of the antioxidant vitamins intakes were significantly correlated with the prevalence of HHcy. After adjusting potential confounders, a linear inverse association between VC intake and HHcy prevalence, and an L-shaped relationship between retinol intake and HHcy prevalence were found, which were not modified by sex or BMI. However, the non-significant effect of VE, carotenes and lutein on HHcy was detected.

Numerous studies have suggested that HHcy may be a modifiable risk factor for CVD, especially for stroke. In the past decades, there were many clinical trials aimed to show the effect of folate and vitamins B_{12} and B_6 supplement on lowering the levels of tHcy, however, the negative results were found in most studies (23). The potential reason may be correlated with the harmful effect of unmetabolized excessive folate or the cyanide and thiocyanate from excessive cyanocobalamin. In China, where folate fortification has not been implemented, folic acid significantly reduced the risk of stroke was observed from the China Stroke Primary Prevention Trial (CSPPT) (24). Thus, appropriate B vitamins therapy is of great importance for lowering tHcy levels in stroke prevention.

Notably, folate, which exists in blood and tissues mainly in a labile form, is very sensitive to oxidative stress (12-14). In addition, methionine synthase and cystathionine β-synthase (the key enzymes involved in methionine and homocysteine metabolism) were strongly influenced by oxidative stress, which may be associated with dietary

antioxidant vitamins and Hcy levels (25, 26). In the past decades, several researches reported the potential association between plasma levels or intake of different antioxidant vitamins and tHcy levels (15, 16, 27-28). In 1999, Brude IR *et al.* observed the inverse association between plasma tHcy concentration and dietary intake of vegetables, vitamin C and β-carotene from 41 participants (27). In addition, dietary intake of retinol equivalents, β-carotene and VC were inversely correlated with plasma tHcy levels, after adjustment for dietary B-vitamins, but not after additional adjustment for plasma folate and vitamin B_{12} (16). What's more, the study focused on the effect of antioxidant vitamins on the plasma tHcy levels in a free-living elderly population found that plasma VC, rather than the intake and supplementation of VC, showed a negative association with tHcy in simple regression analysis, and also found that the plasma levels, as well as the intake and supplementation of vitamin E, and β-carotene were not associated with tHcy (15). Similarly, the cross-sectional NHANES 1999–2002 study found that dietary VC and VE intake were associated with a lower prevalence of elevated blood tHcy concentration, whereas no association between dietary carotenes intake and tHcy was detected (28).

Consistent with our findings, these studies have a common conclusion that VC intake was inversely correlated with tHcy levels. Given the report of an interaction of VC and folate (29), Magana AA *et al.* found the underlying molecular mechanisms that VC activates the folate-mediated one-carbon cycle in C2C12 myoblasts (30). Thus, VC has been explored as an attractive factor to increase circulating levels of folic acid and to reduce Hcy levels.

We found an L-shaped association between dietary retinol intake and high tHcy prevalence, which meant that if the retinol intake was low, the risk of HHcy was decreased as retinol intake increased, but the risk was not changed when retinol intake reached certain level, which was more than 147.2 μg/d among the participants in this study. The dietary intake of retinol equivalents was inversely correlated with plasma tHcy levels after adjustment for dietary B-vitamins (16). Retinol, a preformed vitamin A, plays an important role in vision, cellular differentiation, and proliferation, as well as the immune system regulation. In addition, there is increasing evidence indicates that

retinol seems to inhibit thrombosis (31) and inflammation effects (32), which indicates retinol is emerging as a factor of interest to CVD. Brazionis L reported that plasma retinol was a novel marker for CVD mortality in Australian adults, with an inverse association between plasma retinol in the middle tertile and 5-year CVD mortality (33). Similarly, a strong association between low retinol and the risk of sudden cardiac death was examined (34). In addition, a nested case-control study showed a significant inverse association between plasma retinol and the risk of first stroke among Chinese hypertensive adults from the CSPPT (35), which may due to relatively low baseline retinol concentrations (median: 67.5 μg/dL). Besides, the interaction of retinol and tHcy on CVD risk was also reported. Yu Y (35) showed the inverse effect between plasma retinol and first stroke was stronger among the participants whose tHcy < 10 μmol/L than whose tHcy ≥ 10 μmol/L. Olsen T (36) found that the plasma tHcy was associated with acute myocardial infarction only in the upper Vit-A tertile, and the potential mechanisms may include inflammation and lipid metabolism, which may be partly interpreted with the high intake of retinol (1576 μg RAE/d).

In addition, the dietary source of retinol may contribute to the non-significant effect of retinol in the highest quartile in the fully adjusted model, as it is present in animal-based foods, particularly in liver and whole milk. In this study, we found the TG level was gradually increased with the increase of retinol (data not shown). High retinol intake may alter lipid metabolism by increasing TG level, which may impact the tHcy metabolism (37).

At present, a few studies demonstrate the complicated relationship between retinol, tHcy and CVD risk, but the underlying mechanism has not yet been clarified (38). The antioxidant activity of retinol may be a plausible mechanism that links the effect of retinol on tHcy levels, as retinol is essential for the maintenance of immune function and antioxidant defence (39, 40).

However, we found no relationship between HHcy prevalence and dietary intake of VE. The aforementioned study has found significant inverse associations between plasma tHcy and dietary intake of VE, but the effect was not significant after adjusting B-vitamins intake, which was consistent with our finding (16). On the contrary, dietary

VE (α-tocopherol) intake was associated with a lower risk of elevated blood tHcy concentration among US adults (28).

Epidemiological studies have reported the positive role of carotenoids on human health. In this study, we focused on the effect of carotenes (β-carotene, α-carotene and β-cryptoxanthin) and lutein on tHcy, however, the negative results were found. Many of the aforementioned studies reported the protective effect of β-carotene by lowering tHcy concentration (16, 27), but the risk of tHcy > 13 μmol/L was associated with the total carotene intake from diet plus supplement use, rather than the only intake from diet (28). The negative finding of lutein cannot be compared because of a lack of previously reported data. Thus, more prospective cohort studies and randomized double trials are warranted to indicate the relationship of antioxidant vitamins with HHcy risk.

The strength of this study is the face-to-face researcher-administered FFQ survey. First, to our knowledge, this is the first study to demonstrate an association of dietary intake of antioxidant vitamins with HHcy prevalence that was conducted in both a male and female hypertensive population. Then, the researcher-administered face-to-face interview was considered by validated FFQ, which was designed to evaluate dietary intake and gave full consideration to eating habits and food nutrient composition in the Chinese population.

Our study has several limitations. First, based on a cross-sectional study design, we cannot draw a conclusion about causality. Second, we adjusted the dietary intake of folate, B_{12}, B_6, rather than plasma concentrations, which may could not eliminate confounding effect. Just like Konstantinova SV (16) reported, the inverse correlation of dietary retinol intake and plasma tHcy disappeared after adjustment for plasma folate and vitamin B_{12}. Then, we investigated the participants whether they took dietary supplements, while the detailed doses were not recorded. Therefore, we are not able to eliminate the possible association between supplement use and HHcy prevalence. Nevertheless, the study reported that there was some potential benefit from the antioxidant supplementation on plasma tHcy concentration (28), which could stabilize the positive results in this study. In addition, although some confounding factors were included in the analysis, other potential confounders may exist. For instance, the

influence of smoking and drinking on HHcy was not assessed because of the lack of information about the status of smoking and drinking.

In conclusion, we found dietary intake of VC and retinol was inversely associated with HHcy prevalence in middle-aged and older adults with hypertension after adjusting the potential confounders. Our findings have provided suggestive evidence of an inverse relationship between certain antioxidant intake and HHcy, which should be the impetus for longitudinal and random control trails to verify the relationship and direction and to elucidate the underlying mechanisms in the future.

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Contributors

The authors' responsibilities were as follows: H.L. conceived and designed the study and critically revised the manuscript. P.X. and G.Q. analyzed the data and wrote the paper. Z.J. participated in the laboratory assay. M.J. and Z.D. collected the data and revised the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

No, there are no competing interests for any author.

Ethics approval

The survey protocol was approved by the Ethics Committee of the Shenzhen Nanshan Centre for Chronic Disease Control, and all participants provided written informed consent before enrolment.

Data availability statement

No data are available.

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Table 1 Basic characteristics of the participants [#]

	Total (n=1465)	HHcy (n=469)	non-HHcy (n=996)	*P*
Age (years)	62.0 ± 10.7	64.4 ± 11.4	60.9 ± 10.2	<0.001
Gender (Male, n (%))	729 (49.8)	339 (72.3)	390 (39.2)	<0.001
BMI (kg/m^2)	24.9 ± 3.5	25.0 ± 3.4	24.8 ± 3.6	0.252
WC (cm)	87.3 ± 9.5	88.7 ± 10.0	86.7 ± 9.2	<0.001
SBP (mmHg)	132.9 ± 13.5	133.9 ± 14.0	132.4 ± 13.3	0.046
DBP (mmHg)	80.4 ± 10.6	81.2 ± 11.4	80.0 ± 10.2	0.050
tHcy (μmol/L)	14.63 ± 9.06	21.57 ± 13.35	11.37 ± 1.89	<0.001
FPG (mmol/L)	5.42 ± 1.54	5.29 ± 1.35	5.49 ± 1.62	0.024
TC (mmol/L)	5.25 ± 1.64	5.20 ± 2.49	5.27 ± 1.02	0.414
TG (mmol/L)	1.95 ± 1.39	2.01 ± 1.34	1.92 ± 1.41	0.254
LDL-C (mmol/L)	3.19 ± 0.87	3.14 ± 0.89	3.21 ± 0.87	0.133
Uric acid (μmol/L)	380 ± 97	420 ± 103	362 ± 89	<0.001
Creatinine (μmol/L)	83 ± 44	98 ± 67	76 ± 25	<0.001
Sedentary time <3h/d (n (%))	667 (45.5)	211 (45.0)	456 (45.8)	0.776
History of disease (n (%))				
Coronary heart disease	178 (12.2)	72 (15.4)	106 (10.6)	0.010
Cerebral hemorrhage	15 (1.0)	11 (2.3)	4 (0.4)	<0.001
Cerebral thrombosis	61 (4.2)	34 (7.2)	27 (2.7)	<0.001
Diabetes	240 (16.4)	82 (17.5)	158 (15.9)	0.434
Kidney disease	57 (3.9)	34 (7.2)	23 (2.3)	<0.001
Medications use (n (%))				
Antihypertensive	1146 (78.2)	380 (81.0)	766 (76.9)	0.075
Antihyperglycemic	21(1.4)	8 (1.7)	13 (1.3)	0.547
Lipid-lowering	21 (1.4)	5 (1.1)	16 (1.6)	0.417

BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HHcy, hyperhomocysteinemia; LDL-C, low density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; tHcy, total homocysteine; WC, waist circumference. [#]: Values are mean ± SD or median (interquartile range) or number (percentage).

Table 2 The dietary intake of food and nutrients of the participants [#]

Dietary intake (/d)	Total (n=1465)	HHcy (n=469)	non-HHcy (n=996)	*P*
Energy (kcal)	1488 (1172, 1899)	1508 (1202-1874)	1473 (1150-1913)	0.460
Vegetable (g)	383.7 (240.3, 578.0)	377.7 (227.2, 580.0)	388.2 (252.5, 577.1)	0.510
Fruit (g)	79.2 (39.7, 139.5)	75.4 (33.2, 127.5)	81.1 (42.1, 144.6)	0.036
Protein (g)	43.2 (35.4, 53.5)	41.7 (33.9-50.9)	44.2 (36.2-54.8)	<0.001
Fat (g)	54.3 (42.2, 65.6)	52.5 (41.6-64.3)	55.1 (42.6-65.9)	0.055
Carbohydrate (g)	206.6 (179.4, 234.4)	212.6 (186.6-237.7)	204.4 (176.6-231.7)	<0.001
Cholesterol (mg)	232.7 (152.3, 325.8)	228.3 (154.5, 313.7)	236.6 (147.3, 333.6)	0.229
Fibre (g)	11.2 (8.2, 15.2)	10.6 (7.9-14.8)	11.4 (8.4-15.2)	0.021
Vitamin C (mg)	153.5 (91.2, 240.9)	138.6 (85.6-231.6)	159.5 (93.6-242.7)	0.072
Vitamin E (mg)	25.0 (19.3-31.9)	24.1 (19.0-31.5)	25.4 (19.5-32.2)	0.154
Carotenes (mg)	3.3 (1.8, 5.6)	3.1 (1.7-5.5)	3.5 (1.8-5.6)	0.228
Retinol (μg)	138.3 (78.0, 283.6)	110.9 (62.2-239.3)	152.0 (83.3-317.6)	<0.001
Lutein (mg)	9.5 (5.2, 14.8)	9.0 (5.1-14.2)	9.6 (5.3-15.0)	0.269
Folate (μg)	170.4 (109.4, 263.5)	160.0 (99.9-246.2)	176.1 (112.8-266.9)	0.009

continued on next page

Table 2 *(continued)*

	Total	HHcy (n=469)	non-HHcy (n=996)	*P*
Vitamin B_6 (mg)	0.3 (0.2, 0.5)	0.3 (0.2-0.5)	0.3 (0.2-0.5)	0.233
Vitamin B_{12} (μg)	2.7 (1.6, 4.6)	2.3 (1.4-4.0)	3.0 (1.8-4.9)	<0.001
Saturated fatty acid (g)	10.6 (8.1-13.2)	10.2 (7.8-12.9)	10.8 (8.3-13.3)	0.016
Monounsaturated fatty acid (g)	12.7 (9.8-16.3)	12.4 (9.7-16.1)	12.9 (9.9-16.3)	0.111
Polyunsaturated fatty acid (g)	20.0 (14.9-26.4)	19.7 (14.9-26.0)	20.2 (15.0-26.7)	0.292
Selenium (μg)	31.3 (23.2, 41.9)	28.6 (21.0-38.8)	32.5 (24.5-43.7)	<0.001
Magnesium (mg)	216.1 (173.3- 269.2)	205.8 (167.7-261.5)	221.0 (176.3-271.6)	0.011
Zinc (mg)	7.0 (6.0- 8.3)	6.8 (5.9-7.9)	7.1 (6.1-8.5)	<0.001
Iron (mg)	16.6 (12.0- 23.4)	16.5 (11.8-23.1)	16.6 (12.1-23.6)	0.295
Supplement use (n (%))	302 (20.6)	80 (17.1)	222 (22.3)	0.021

[#]: Values are median (interquartile range) or number (percentage). HHcy, hyperhomocysteinemia.

Table 3 β (95% CIs) and ORs (95% CIs) of dietary intakes of antioxidant vitamins and homocysteine levels among the middle-aged and older hypertensive participants

tHcy		Q1	Q2	Q3	Q4	$P_{\text{for trend}}$
Vitamin C (mg/d)		< 91.2	91.2-153.5	153.6-240.9	≥ 240.9	
Cases (%)		132 (36.1)	122 (33.3)	104 (28.4)	111 (30.2)	
Crude Model	-0.037 (-0.063, -0.011)	Ref.	0.89 (0.65, 1.20)	0.70 (0.52, 0.96)	0.77 (0.56, 1.05)	0.071
Model I	-0.031 (-0.055, -0.006)	Ref.	0.92 (0.66, 1.27)	0.64 (0.46, 0.90)	0.55 (0.34, 0.89)	0.048
Model II	-0.050 (-0.084, -0.016)	Ref.	0.82 (0.57, 1.16)	0.49 (0.33, 0.74)	0.40 (0.22, 0.74)	0.001
Vitamin E (mg/d)		< 19.3	19.3-25.0	25.1-31.9	≥ 31.9	
Cases (%)		122 (33.6)	126 (34.2)	113 (30.9)	108 (29.3)	
Crude Model	-0.035 (-0.084, 0.014)	Ref.	1.03 (0.76, 1.40)	0.88 (0.65, 1.20)	0.82 (0.60, 1.12)	0.139
Model I	-0.007 (-0.053, 0.039)	Ref.	1.10 (0.79, 1.53)	0.96 (0.69, 1.34)	0.95 (0.68, 1.33)	0.618
Model II	-0.009 (-0.055, 0.036)	Ref.	1.12 (0.80, 1.57)	0.95 (0.68, 1.33)	0.91 (0.65, 1.28)	0.412
Carotenes (mg/d)		< 1.78	1.78-3.30	3.31-5.61	≥ 5.61	
Cases (%)		118 (32.7)	136 (37.0)	99 (26.8)	116 (31.6)	
Crude Model	-0.024 (-0.045, -0.003)	Ref.	1.21 (0.89, 1.64)	0.76 (0.55, 1.04)	0.95 (0.70, 1.30)	0.315

continued on next page

Table 3 (*continued*)

	tHcy	Q1	Q2	Q3	Q4	$P_{\text{for trend}}$
Model I	-0.022 (-0.042, -0.003)	Ref.	1.22 (0.88, 1.69)	0.72 (0.51, 1.01)	0.93 (0.67, 1.30)	0.254
Model II	-0.022 (-0.052, 0.009)	Ref.	1.18 (0.84, 1.68)	0.68 (0.46, 1.02)	0.76 (0.43, 1.35)	0.159
Retinol (μg/d)		< 78.0	78.0-138.3	138.4-283.6	≥ 283.6	
Cases (%)		147 (40.2)	133 (36.3)	91 (24.9)	98 (26.7)	
Crude Model	-0.030 (-0.046, -0.015)	Ref.	0.85 (0.63, 1.15)	0.49 (0.36, 0.68)	0.54 (0.40, 0.74)	0.001
Model I	-0.027 (-0.041, -0.012)	Ref.	0.84 (0.61, 1.16)	0.50 (0.35, 0.70)	0.55 (0.40, 0.77)	0.003
Model II	-0.021 (-0.041, -0.002)	Ref.	0.90 (0.65, 1.25)	0.61 (0.42, 0.86)	0.86 (0.56, 1.32)	0.951
Lutein (mg/d)		< 5.22	5.22-9.48	9.49-14.82	≥ 14.82	
Cases (%)		123 (33.7)	120 (32.8)	114 (31.1)	112 (30.4)	
Crude Model	-0.028 (-0.053, -0.004)	Ref.	0.96 (0.71, 1.31)	0.89 (0.65, 1.21)	0.86 (0.63, 1.17)	0.311
Model I	-0.024 (-0.046, -0.001)	Ref.	0.98 (0.71, 1.37)	0.84 (0.61, 1.17)	0.87 (0.62, 1.22)	0.331
Model II	-0.025(-0.063, 0.013)	Ref.	0.92 (0.65, 1.31)	0.76 (0.51, 1.14)	0.66 (0.37, 1.19)	0.138

Model I: Adjusted for age, sex ('male' as the reference) BMI.
Model II: Adjusted for variables in Model I and further adjusted for sedentary time ('< 3 h/d' as the reference), the intakes of folate, vitamin B6 and vitamin B12, supplement use ('no' as the reference) and the history of cardiovascular events ('no' as the reference).

Figure Legends:

Fig. 1 Restricted cubic spline analyses illustrating the shapes of multivariable association between dietary vitamin C intake (A), or dietary retinol intake (B) and HHcy. Adjusted for age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin B_6 and vitamin B_{12}, the history of cardiovascular events ('no' as the reference).

Fig. 2 ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to vitamin C, retinol intake in subgroups, stratified by sex (A), BMI (B). Adjusted for age, sex (except sex subgroup), BMI (except BMI subgroup), sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin B6 and vitamin B12, the history of cardiovascular events ('no' as the reference).

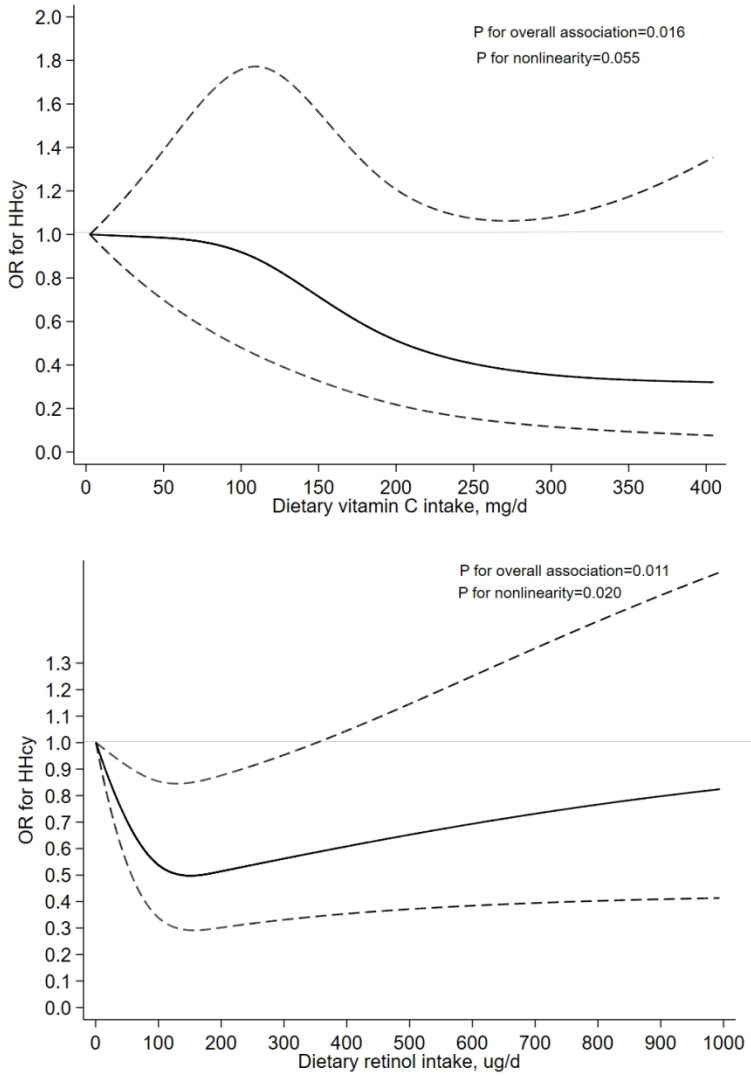


Figure 1 Restricted cubic spline analyses illustrating the shapes of multivariable association between dietary vitamin C intake (A), or dietary retinol intake (B) and HHcy. Adjusted for age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin B6 and vitamin B12, the history of cardiovascular events ('no' as the reference).

187x287mm (144 x 144 DPI)

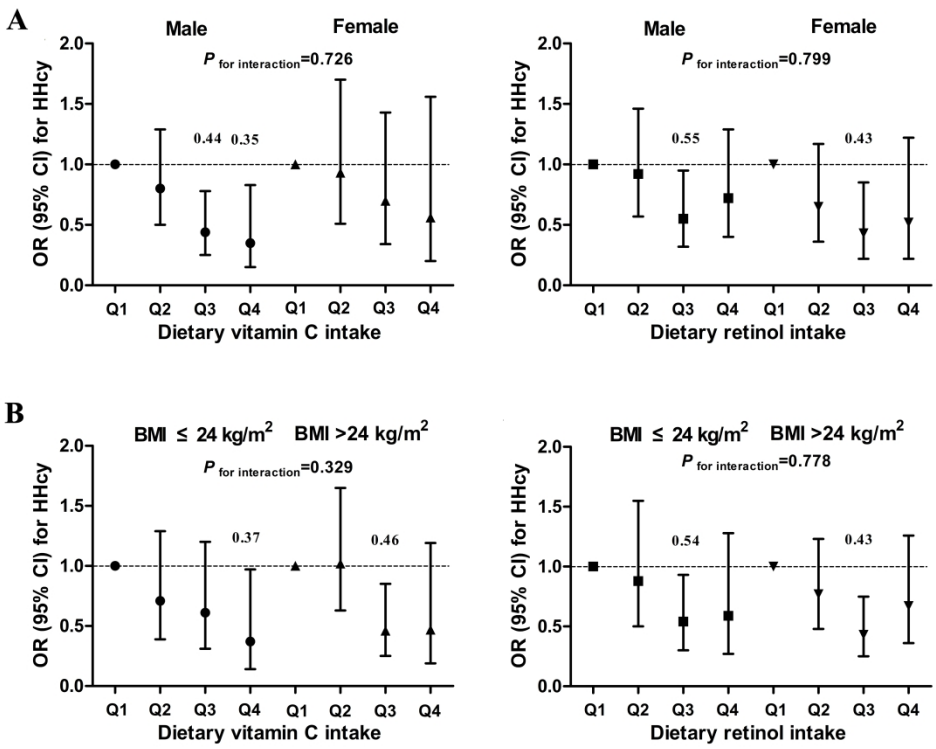
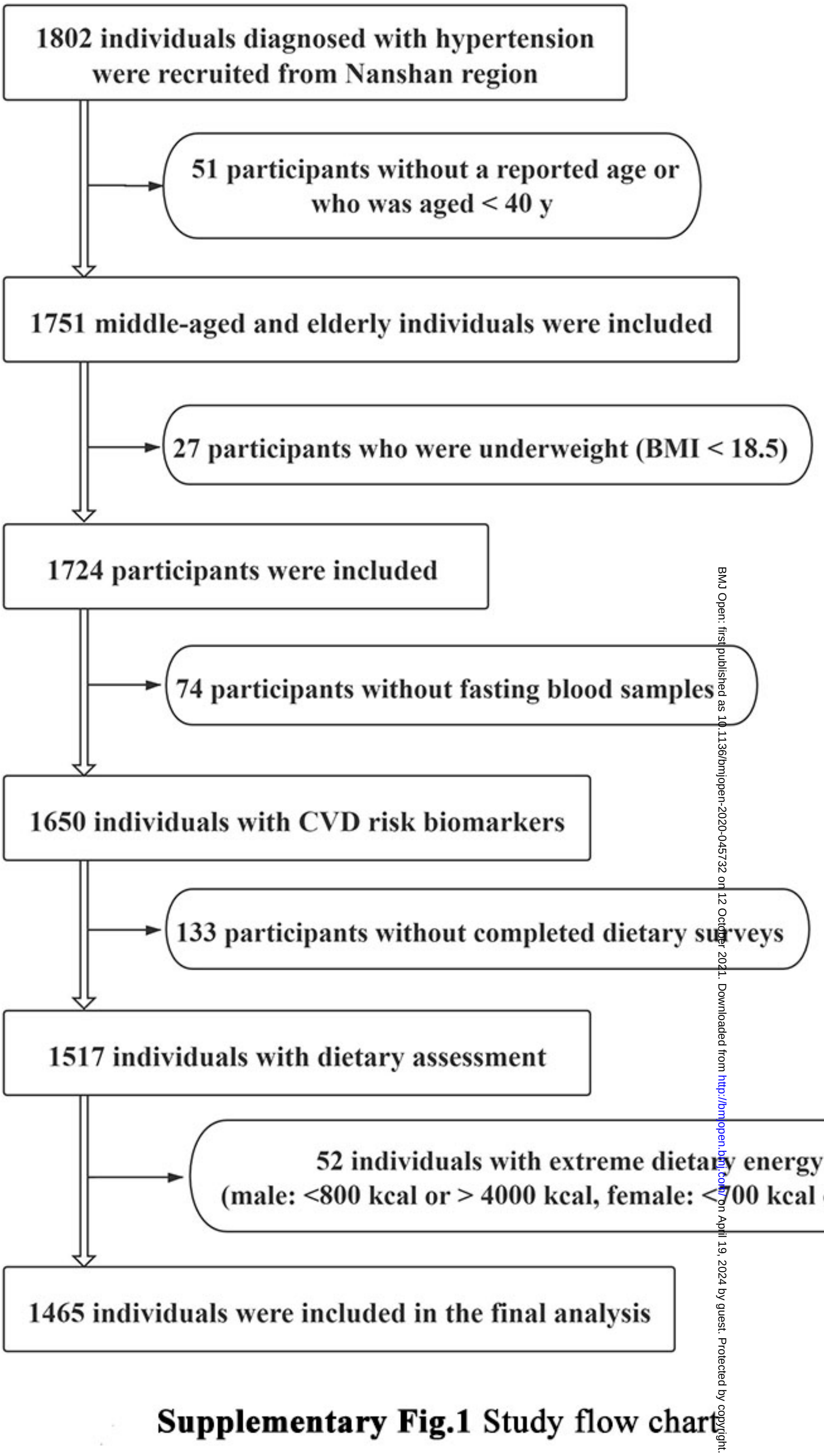
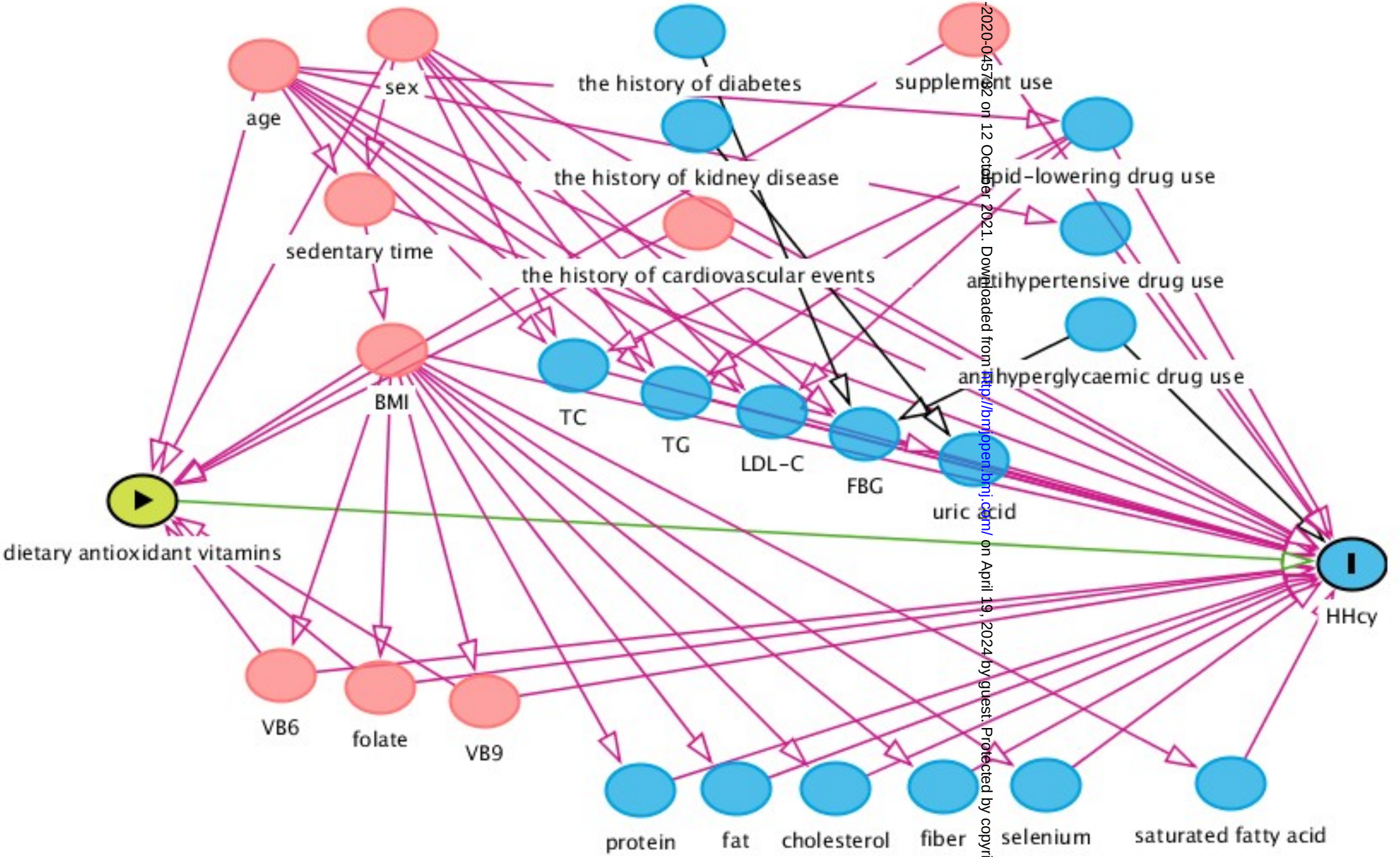


Figure 2 ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to vitamin C, retinol intake in subgroups, stratified by sex (A), BMI (B). Adjusted for age, sex (except sex subgroup), BMI (except BMI subgroup), sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin B6 and vitamin B12, the history of cardiovascular events ('no' as the reference).



Supplementary Fig.1 Study flow chart



Supplementary Figure 2 Directed acyclic graph drawn in the DAGitty programme to identify the minimum set of confounders (depicted in red) to enter the adjusted model to examine the association between dietary intakes of antioxidant vitamins and HHcy

Supplementary Table 1 Sensitivity analyses of ORs (95% CIs) of antioxidant vitamins intake for hyperhomocysteinemia prevalence in multivariable-adjusted model among the patients with hypertension [#]

	Q1	Q2	Q3	Q4	$P_{\text{for trend}}$
Without cardiovascular events (n=1245)					
Vitamin C	1.00 (Ref.)	0.92 (0.63, 1.35)	0.54 (0.35, 0.84)	0.41 (0.21, 0.80)	0.003
Vitamin E	1.00 (Ref.)	1.14 (0.79, 1.64)	0.95 (0.66, 1.39)	0.90 (0.62, 1.31)	0.425
Carotenes	1.00 (Ref.)	1.25 (0.85,1.84	0.67 (0.43, 1.04)	0.77 (0.41, 1.44)	0.178
Retinol	1.00 (Ref.)	1.05 (0.73, 1.50)	0.61 (0.43, 0.94)	0.78 (0.50, 1.34)	0.836
Lutein	1.00 (Ref.)	1.04 (0.71, 1.53)	0.74 (0.48, 1.14)	0.63 (0.33, 1.20)	0.104
Without tHcy-lowering drug-using (n=1360)					
Vitamin C	1.00 (Ref.)	0.85 (0.58, 1.24)	0.49 (0.31, 0.78)	0.44 (0.22, 0.86)	0.007
Vitamin E	1.00 (Ref.)	1.07 (0.74, 1.55)	0.98 (0.68, 1.42)	0.95 (0.66, 1.37)	0.685
Carotenes	1.00 (Ref.)	1.27 (0.87, 1.85)	0.69 (0.44, 1.07)	0.67 (0.35, 1.28)	0.090
Retinol	1.00 (Ref.)	0.84 (0.59, 1.20)	0.56 (0.38, 0.84)	0.85 (0.54, 1.35)	0.837
Lutein	1.00 (Ref.)	0.88 (0.60, 1.29)	0.74 (0.47, 1.15)	0.60 (0.31, 1.16)	0.115

[#]: Adjusted for age, sex, BMI, sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin B_6 and vitamin B_{12}.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4-5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	6-7
		(*c*) Explain how missing data were addressed	6-7
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	6-7
		(*e*) Describe any sensitivity analyses	6-7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	5
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7-8
		(b) Indicate number of participants with missing data for each variable of interest	7-8
Outcome data	15*	Report numbers of outcome events or summary measures	7-8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-9
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12-13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association between dietary antioxidant vitamins intake and homocysteine levels in middle-aged and older adults with hypertension: a cross-sectional study

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Association between dietary antioxidant vitamins intake and homocysteine levels in middle-aged and older adults with hypertension: a cross-sectional study

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Abstract

Objectives: Plasma total homocysteine (tHcy) has been implicated in the development of cardiovascular disease. This study aimed to assess the relationship of dietary antioxidant vitamins intake with tHcy levels in middle-aged and older adults with hypertension.

Design: A cross-sectional study.

Setting: The survey was conducted in the Nanshan district of Shenzhen.

Participants: A total of 1465 middle-aged and older adults with hypertension were included between July and September of 2013.

Measurements: Hyperhomocysteinemia (HHcy) was defined as tHcy ≥ 15 μmol/L. Some dietary antioxidant vitamins (vitamin C (VC) and vitamin E (VE), carotenes, retinol, lutein) intake was estimated using the food frequency questionnaire. Socio-demographic and potential covariates were evaluated through questionnaires, anthropometric measurements and blood tests. The association between dietary intakes of antioxidant vitamins and tHcy concentration were evaluated by multiple linear regression analyses after napierian logarithm -transformed. Multiple logistic regression models were further used to determine odds ratios (ORs) and 95% confidence intervals (CIs).

Results: The β (95% CIs) of VC intake and tHcy was -0.050 (-0.084, -0.016). Compared with the lowest quartile in the fully adjusted model, the ORs (95% CIs) for HHcy levels across quartiles of dietary VC intake were 0.82 (0.57, 1.16), 0.49 (0.33, 0.74) and 0.40 (0.22, 0.74) (*P* for trend=0.001). The β (95% CIs) of retinol intake and tHcy was -0.021 (-0.041, -0.002), and the ORs (95% CIs) in the third quartile of retinol intake was 0.61 (0.42, 0.86), while the effect for the highest quartile was not significant (*P* for trend=0.951). No significant association was observed between dietary VE, carotenes and lutein intake and HHcy.

Conclusions: A linear inverse association between dietary VC intake and HHcy prevalence, and an L-shaped association between dietary retinol intake and HHcy prevalence were found in Chinese middle-aged and older adults with hypertension.

Strengths and limitations of this study:

1. This study focused on the risk of hyperhomocysteinemia among middle-aged and older adults with hypertension.
2. A linear inverse association between dietary VC intake and HHcy prevalence and the threshold effect of retinol on HHcy was reported in this study.
3. Based on a cross-sectional study design, we could only draw a conclusion about correlation, not causation.
4. Although some confounding factors were included in the analysis, other potential confounders may exist.

Introduction

Increasing evidence has shown that elevated total homocysteine (tHcy) levels are associated with atherosclerotic cardiovascular diseases, stroke, peripheral arterial occlusive disease, and venous thrombosis (1, 2). Hyperhomocysteinemia (HHcy) (tHcy $\geq$ 15 μmol/L) (3), the result of a disturbed methionine metabolism, may lead to an enhancement of the adverse effects of risk factors like hypertension on human health (4). Hypertension is a major risk factor for cardiovascular and chronic kidney disease and a major cause of the global burden of disease and mortality (5, 6). It was estimated that the prevalence of hypertension in China in 2015 was 23.2%, and about 245 million Chinese adults had hypertension (7). Notably, the incidence of hypertension with hyperhomocysteinemia, or 'H-type hypertension', is significantly higher compared with other countries, representing 75% of Chinese patients with hypertension (8). Thus, the nutritional treatment focus on the attempt to reduce cardiovascular risk by reducing tHcy is important, particularly among patients with hypertension (9).

Increasing age, male sex, genetic factors, consumption of alcohol or tobacco, kidney function and physical activity are some of the factors associated with tHcy levels (10). Epidemiological studies and clinical trials have indicated that folate, vitamin B_{12} and vitamin B_6 status, well-known predictors of tHcy, are important for tHcy metabolism. The latest meta-analysis demonstrated that a lower risk of stroke and overall cardiovascular disease (CVD) with folic acid supplementation, which may partly

contribute to the decrease of tHcy levels (11).

Folic acid and vitamin B_{12}, which are involved in remethylation of homocysteine to methionine by methionine synthase (MS), and vitamin B_6, which acts as a cofactor in the transsulfuration of homocysteine to cysteine by cystathionine β-synthase (CBS) (12). Folate, a key factor of tHcy metabolism, is very sensitive to free radicals (13-15). In addition, MS and CBS were strongly influenced by oxidative stress, which may be associated with dietary antioxidant vitamins and Hcy levels (16,17). We hypothesized that antioxidant nutrients may regulate tHcy metabolism by influencing methionine-homocysteine cycle. Plasma levels or dietary intake of vitamin C (VC), vitamin E (VE) or β-carotene was inversely associated with tHcy levels, however, the findings were not consistent (18-20). Of note, the association has never been investigated among the hypertensive population.

Therefore, this large population-based study aimed to determine the association between the dietary intake of VC, VE, carotenes, retinol and lutein and the prevalence of HHcy in middle-aged and older men and women with hypertension.

Methods

Study design and population

This study consecutively recruited individuals with hypertension from 60 community health service centres (CHSCs) in the Nanshan district of Shenzhen from July to September of 2013 using a three-stage random sampling method. In the first stage, 8 sub-districts were selected in the Nanshan district; in the second stage, 6 to 8 communities were selected from each sub-district using a simple random selection procedure; and in the third stage, individuals with hypertension were selected from each community using isometric random sampling. All subjects were of Chinese ethnicity and had lived in the Nanshan district of Shenzhen for over six months. The individuals were invited to visit the CHSCs, where the researcher-administered questionnaire (including the validated food frequency questionnaire (FFQ)) was conducted, the anthropometric measurements were recorded, and fasting blood samples were collected.

We collected the data of 1802 participants, and excluded 51 participants whose age was not reported or who were aged ≤ 40 years and 27 underweight participants (body

mass index (BMI) < 18.5 kg/m^2), as well as participants for whom fasting blood samples (n = 74) and complete dietary surveys (n = 133) were not available. We also excluded participants with an extreme dietary energy intake (male: < 800 kcal or > 4000 kcal, female: < 700 kcal or > 3500 kcal) (n = 52). Finally, 1465 participants were included in the analysis **(Supplementary Figure 1)**.

Patient and public involvement

The development of standardised form is in response to the public health need of preventing stroke among hypertension population. Patients and the public were not involved in the design of the study. The results of our study will be disseminated through open access publications.

Dietary assessment

The researcher-administered FFQ consisted of 92 food items, which were assembled into 7 food groups: cereals, vegetables, fruits, beans, animal foods, nuts and beverages. The FFQ was based on the national FFQ used in the 2010-2012 China National Nutrition and Health Survey according to the Chinese Nutrition and Health Surveillance in 2010-2012 (21). Participants were asked to recall the consumption of each item during the past year, including the type of food, frequency and amount. Food weight maps were available for participants to estimate their portion size. Primary data obtained from FFQs were doubly entered into the EpiData 3.0 software by two trained technicians to verify accuracy. Dietary energy and other nutrients were calculated based on the Chinese Food Composition Database (22, 23). Dietary intake of VC, VE, carotenes (consist of α-carotene, β-carotene and β-cryptoxanthin), and retinol were calculated based on the Chinese Food Composition Table 2002 (22). We report carotenes and retinol separately, because we could not get the accurate calculation for vitamin A, as the data for carotenes were combined with different carotene, rather than separate one. Dietary intake of lutein was calculated based on the food composition table of vegetables, fruits, eggs and nuts that contain large amounts of lutein, according to Chinese Dietary Reference Intakes 2013 (24). The intake of all dietary nutrients and carotenoids was adjusted for energy using the residual method (25). The second FFQ was conducted 3 weeks after the completion of the first FFQ among 108 participants.

The intra-class correlation coefficients of two administrations of FFQ for nutrients of VC, VE, carotenes, retinol, lutein were 0.395, 0.477, 0.355, 0.551 and 0.350, and were all statistically significant.

Assessment of other covariates

Participants' socio-demographic characteristics (e.g., age and sex), lifestyle factors (e.g., sedentary time), history of chronic diseases, and medication and supplement use status were collected. Sedentary time consisted of time spent watching TV and sitting, which were combined into one variable with 2 categories, < 3 h/d and ≥ 3 h/d, based on a median sedentary time of 3 h/d. The history of cardiovascular events including coronary heart disease, cerebral haemorrhage, cerebral thrombosis, and the history of diabetes and kidney disease were recorded for each participant. The prescription use status was classified into 2 groups (yes or no) corresponding to whether the participant was taking any type or quantity of drugs, including antihypertensive drugs, antihyperglycaemic drugs, lipid-lowering drugs or tHcy-lowering drugs. Supplement use consisted of vitamin A, retinol, B vitamins, VC, VE and folic acid was included.

Anthropometric measurements

Height and weight and waist circumference (WC) were measured by specialists. BMI was calculated as weight (kg) / height (m^2). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured from the right arm of participants in a seated position after a sufficient rest period using a mercury sphygmomanometer in the morning. Blood pressure was measured manually and recorded as the average of three measurements.

Laboratory tests and outcomes

Morning fasting elbow vein blood samples were required to fast overnight (at least 10 hours) were collected from the participants at the CHSCs and transported under refrigerated conditions to a clinical laboratory of the Nanshan Centre for Chronic Disease Control within 2 hours. The blood specimens were collected in a 5-ml EDTA vacuum tube. Blood samples were collected through deposition and centrifugation for ten minutes at 3000 r/min at room temperature. The concentrations of plasma tHcy, fasting plasma glucose (FPG), total cholesterol (TC), triglyceride (TG), low-density

lipoprotein cholesterol (LDL-C), uric acid and creatinine were assessed on the day of blood collection using enzymatic methods via an auto-analyser (HITACHI 7080). The inter-day quality control assessments met the standard during the analysis. HHcy was defined as plasma tHcy concentration ≥ 15 μmol/L.

Statistical analysis

Demographic characteristics were described by means ± SDs for normally distributed data, medians (interquartile ranges, IQRs) for non-normally distributed data and numbers (percentages) for categorical data. The differences between males and females were compared using the *t* test for normally distributed variables, the Kruskal-Wallis rank test for non-normally distributed variables, and the chi-square test for categorical variables.

Both dietary intakes of antioxidant vitamins and tHcy concentration were napierian logarithm (ln)-transformed to improve normality before analyses and categorised into quartiles. The associations between dietary antioxidant vitamins intake and the prevalence of HHcy were analyzed using multiple logistic regression models, with the lowest quartile as the reference category. Confounding variables were selected based on the minimal sufficient adjustment recommended by the Directed Acyclic Graph, created in the online software Dagitty 3.0 (**Supplementary Figure 2**). The selected potential confounders included age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as the reference), the intakes of folate, vitamin B_6 and vitamin B_{12}, supplement use ('no' as the reference) and the history of cardiovascular events ('no' as the reference). Linear trends were tested by creating a continuous variable for dietary antioxidant vitamins intake using the median value for each quartile. The sensitive analyses between dietary antioxidant vitamins intake and HHcy prevalence were applied among the population who had never suffered cardiovascular events, or among the population who never use the tHcy-lowering drug.

To further explore the nonlinearity of the relationship between dietary antioxidant vitamins intake and high tHcy, we used restricted cubic spline with 4 knots at the 5^{th}, 35^{th}, 65^{th} and 95^{th} percentiles of dietary antioxidant vitamins intake.

To evaluate the modification effect by some potential prevalence factors of HHcy,

including sex (male or female) and BMI (<24 or ≥24 kg/m^2), stratified analyses were conducted by these potential factors and estimated *P* values for interaction terms.

All data were analyzed using the R Foundation version 3.1.2 and Empower X&Y Solutions Inc. Statistical significance was considered when $P < 0.05$ (two-sided).

Results

In this cross-sectional study, 1465 participants (male: 729, female: 736) were included in the analysis, and the mean ± SD of age and BMI was 62.0 ± 10.7 years, and 24.9 ± 3.5 kg/m^2. The tHcy levels of the participants was 14.63 ± 9.06 μmol/L, and the number (percentage) of HHcy was 469 (32.0%). The participants with HHcy were older and more male, and have higher WC, SBP, tHcy, uric acid, creatinine, and higher percentages of history of cardiovascular events (including coronary heart disease, cerebral hemorrhage, cerebral thrombosis) and kidney disease, and have lower FPG level (all $P < 0.05$) (**Table 1**).

The dietary intakes of nutrients were shown in **Table 2**. The median (IQR) of dietary antioxidant vitamins intake were as follows: VC 153.5 (91.2, 240.9) mg/d, VE 25.0 (19.3, 31.9) mg/d, carotenes 3.3 (1.8, 5.6) mg/d, retinol 138.3 (78.0, 283.6) μg/d, and lutein 9.5 (5.2, 14.8) mg/d. Among the participants with HHcy, the intake of carbohydrate was higher, and the intake of fruit, protein, fibre, retinol, folate, vitamin B_{12}, saturated fatty acid, selenium, magnesium, zinc was lower, and the percentage of supplement use was lower (all $P < 0.05$).

The association between dietary antioxidant vitamins intake and tHcy levels are shown in **Table 3**. The inverse association between VC intake and tHcy concentration after ln-transformed in the fully adjusted model, and the β (95% CIs) was -0.050 (-0.084, -0.016), which was consistent with the logistic regression as categorical variables. In Model II, the significant association was found in the third and highest quartile of VC intake, and the ORs (95% CIs) were 0.49 (0.33, 0.74) and 0.40 (0.22, 0.74) ($P_{\text{for trend}} = 0.001$). The retinol intake was also inversely associated with tHcy, as the β (95% CIs) was -0.021 (-0.041, -0.002). After adjusting for age, sex and BMI, the ORs (95% CIs) for HHcy prevalence across quartiles of retinol intake were 1.00, 0.84 (0.61, 1.16), 0.50 (0.35, 0.70), and 0.55 (0.40, 0.77) ($P_{\text{for trend}} = 0.003$). However, in

Model II, the significant association was only found in the third quartile of retinol intake, and the ORs (95% CIs) was 0.61 (0.42, 0.86) ($P_{\text{for trend}}$ =0.951). The non-significant association between dietary intake of VE, carotenes and lutein and HHcy prevalence was found. The results were similar in the sensitivity analyses that excluded participants with cardiovascular events or who use the tHcy-lowering drug (see **Supplementary Table 1**).

After fully adjusting for the potential confounders, the association between dietary antioxidant vitamins intake and the prevalence of HHcy were shown in **Figure.1**. From the cubic splines, we noted that the linear inverse trend of VC intake and HHcy prevalence (*P* for overall association=0.016, *P* for nonlinearity=0.055) and the L-shaped relationship of retinol intake and HHcy prevalence (*P* for overall association=0.011, *P* for nonlinearity=0.020), which were consistent with the results of logistic regression analyses. The threshold analysis showed that the ORs (95% CIs) of HHcy was 0.995 (0.991, 0.995) (*P*=0.005) when retinol intake was lower than 147.2 μg/d and 1.000 (1.000, 1.001) (*P*=0.094) when retinol intake was more than 147.2 μg/d, and the *P* value of log-likelihood ratio was 0.003. The non-association between carotenes, lutein and VE and HHcy was not shown.

In stratified analyses, the association between dietary VC and retinol intake and HHcy prevalence were not significantly modified by sex (male or female), BMI (<24 or ≥24 kg/m^2) (all $P_{\text{for interaction}}$ were >0.05) (**Figure 2**). Similar results of stratified analyses of carotenes, lutein and VE were not shown.

Discussion

In this community-based cross-sectional study, we observed some of the antioxidant vitamins intakes were significantly correlated with the prevalence of HHcy. After adjusting potential confounders, a linear inverse association between VC intake and HHcy prevalence, and an L-shaped relationship between retinol intake and HHcy prevalence were found, which were not modified by sex or BMI. However, the non-significant effect of VE, carotenes and lutein on HHcy was detected.

Numerous studies have suggested that HHcy may be a modifiable risk factor for CVD, especially for stroke. In the past decades, there were many clinical trials aimed

to show the effect of folate and vitamins B_{12} and B_6 supplement on lowering the levels of tHcy, however, the negative results were found in most studies (26). The potential reason may be correlated with the harmful effect of unmetabolized excessive folate or the cyanide and thiocyanate from excessive cyanocobalamin. In China, where folate fortification has not been implemented, folic acid significantly reduced the risk of stroke was observed from the China Stroke Primary Prevention Trial (CSPPT) (27). Thus, appropriate B vitamins therapy is of great importance for lowering tHcy levels in stroke prevention.

In the past decades, several researches reported the potential association between plasma levels or intake of different antioxidant vitamins and tHcy levels (18, 19, 28-30). In 1999, Brude IR *et al.* observed the inverse association between plasma tHcy concentration and dietary intake of vegetables, vitamin C and β-carotene from 41 participants (28). In addition, dietary intake of retinol equivalents, β-carotene and VC were inversely correlated with plasma tHcy levels, after adjustment for dietary B-vitamins, but not after additional adjustment for plasma folate and vitamin B_{12} (19). What's more, the study focused on the effect of antioxidant vitamins on the plasma tHcy levels in a free-living elderly population found that plasma VC, rather than the intake and supplementation of VC, showed a negative association with tHcy in simple regression analysis, and also found that the plasma levels, as well as the intake and supplementation of vitamin E, and β-carotene were not associated with tHcy (18). Similarly, the cross-sectional NHANES 1999–2002 study found that dietary VC and VE intake were associated with a lower prevalence of elevated blood tHcy concentration, whereas no association between dietary carotenes intake and tHcy was detected (29). In addition, Rajesh Ullegaddi et al. found that significant reductions in plasma homocysteine in the group with antioxidant treatment (vitamins C and E) combination with B-vitamin, compared with the group with B-vitamin alone (30), which support the importance of antioxidant vitamins on tHcy metabolism.

Consistent with our findings, these studies have a common conclusion that VC intake was inversely correlated with tHcy levels. Given the report of an interaction of VC and folate (31), Magana AA *et al.* found the underlying molecular mechanisms that VC

activates the folate-mediated one-carbon cycle in C2C12 myoblasts (32). Thus, VC has been explored as an attractive factor to increase circulating levels of folic acid and to reduce Hcy levels.

We found an L-shaped association between dietary retinol intake and high tHcy prevalence, which meant that if the retinol intake was low, the risk of HHcy was decreased as retinol intake increased, but the risk was not changed when retinol intake reached certain level, which was more than 147.2 μg/d among the participants in this study. The dietary intake of retinol equivalents was inversely correlated with plasma tHcy levels after adjustment for dietary B-vitamins (19). Retinol, a preformed vitamin A, plays an important role in vision, cellular differentiation, and proliferation, as well as the immune system regulation. In addition, there is increasing evidence indicates that retinol seems to inhibit thrombosis (33) and inflammation effects (34), which indicates retinol is emerging as a factor of interest to CVD. Brazionis L *et al.* reported that plasma retinol was a novel marker for CVD mortality in Australian adults, with an inverse association between plasma retinol in the middle tertile and 5-year CVD mortality (35). Similarly, a strong association between low retinol and the risk of sudden cardiac death was examined (36). In addition, a nested case-control study showed a significant inverse association between plasma retinol and the risk of first stroke among Chinese hypertensive adults from the CSPPT (37), which may due to relatively low baseline retinol concentrations (median: 67.5 μg/dL). Besides, the interaction of retinol and tHcy on CVD risk was also reported. Yu Y *et al.* (37) showed the inverse effect between plasma retinol and first stroke was stronger among the participants whose tHcy < 10 μmol/L than whose tHcy ≥ 10 μmol/L. Olsen T *et al.* (38) found that the plasma tHcy was associated with acute myocardial infarction only in the upper Vit-A tertile, and the potential mechanisms may include inflammation and lipid metabolism, which may be partly interpreted with the high intake of retinol (1576 μg RAE/d).

In addition, the dietary source of retinol may contribute to the non-significant effect of retinol in the highest quartile in the fully adjusted model, as it is present in animal-based foods, particularly in liver and whole milk. In this study, we found the TG level was gradually increased with the increase of retinol (data not shown). High retinol

intake may alter lipid metabolism by increasing TG level, which may impact the tHcy metabolism (39).

At present, a few studies demonstrate the complicated relationship between retinol, tHcy and CVD risk, but the underlying mechanism has not yet been clarified (40). The antioxidant activity of retinol may be a plausible mechanism that links the effect of retinol on tHcy levels, as retinol is essential for the maintenance of immune function and antioxidant defence (41, 42).

However, we found no relationship between HHcy prevalence and dietary intake of VE. The aforementioned study has found significant inverse associations between plasma tHcy and dietary intake of VE, but the effect was not significant after adjusting B-vitamins intake, which was consistent with our finding (19). On the contrary, dietary VE (α-tocopherol) intake was associated with a lower risk of elevated blood tHcy concentration among US adults (29).

Epidemiological studies have reported the positive role of carotenoids on human health. In this study, we focused on the effect of carotenes (β-carotene, α-carotene and β-cryptoxanthin) and lutein on tHcy, however, the negative results were found. Many of the aforementioned studies reported the protective effect of β-carotene by lowering tHcy concentration (19, 28), but the risk of tHcy > 13 μmol/L was associated with the total carotene intake from diet plus supplement use, rather than the only intake from diet (29). The negative finding of lutein cannot be compared because of a lack of previously reported data. Thus, more prospective cohort studies and randomized double trials are warranted to indicate the relationship of antioxidant vitamins with HHcy risk.

The strength of this study is the face-to-face researcher-administered FFQ survey. First, to our knowledge, this is the first study to demonstrate an association of dietary intake of antioxidant vitamins with HHcy prevalence that was conducted in both a male and female hypertensive population. Then, the researcher-administered face-to-face interview was considered by validated FFQ, which was designed to evaluate dietary intake and gave full consideration to eating habits and food nutrient composition in the Chinese population.

Our study has several limitations. First, based on a cross-sectional study design, we

cannot draw a conclusion about causality. The strong effects observed in this study may be enhanced because of selection effects, because hypertensive individuals may have made changes to their diet in response to diagnosis. Second, we adjusted the dietary intake of folate, B_{12}, B_6, rather than plasma concentrations, which may could not eliminate confounding effect. Just like Konstantinova SV (19) reported, the inverse correlation of dietary retinol intake and plasma tHcy disappeared after adjustment for plasma folate and vitamin B_{12}. Then, we investigated the participants whether they took dietary supplements, while the detailed doses were not recorded. Therefore, we are not able to eliminate the possible association between supplement use and HHcy prevalence. Nevertheless, the study reported that there was some potential benefit from the antioxidant supplementation on plasma tHcy concentration (29), which could stabilize the positive results in this study. In addition, although some confounding factors were included in the analysis, other potential confounders may exist. For instance, the influence of smoking and drinking on HHcy was not assessed because of the lack of information about the status of smoking and drinking.

In conclusion, we found dietary intake of VC and retinol was inversely associated with HHcy prevalence in middle-aged and older adults with hypertension after adjusting the potential confounders. Our findings have provided suggestive evidence of an inverse relationship between certain antioxidant intake and HHcy, which should be the impetus for longitudinal and random control trails to verify the relationship and direction and to elucidate the underlying mechanisms in the future.

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Contributors

The authors' responsibilities were as follows: H.L. conceived and designed the study and critically revised the manuscript. P.X. and G.Q. analyzed the data and wrote the paper. Z.J. participated in the laboratory assay. M.J. and Z.D. collected the data and revised the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

No, there are no competing interests for any author.

Ethics approval

The survey protocol was approved by the Ethics Committee of the Shenzhen Nanshan Centre for Chronic Disease Control (ID: ll20190003), and all participants provided written informed consent before enrolment.

Data availability statement

No data are available.

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Table 1 Basic characteristics of the participants [#]

	Total (n=1465)	HHcy (n=469)	non-HHcy (n=996)	*P*
Age (years)	62.0 ± 10.7	64.4 ± 11.4	60.9 ± 10.2	<0.001
Gender (Male, n (%))	729 (49.8)	339 (72.3)	390 (39.2)	<0.001
BMI (kg/m^2)	24.9 ± 3.5	25.0 ± 3.4	24.8 ± 3.6	0.252
WC (cm)	87.3 ± 9.5	88.7 ± 10.0	86.7 ± 9.2	<0.001
SBP (mmHg)	132.9 ± 13.5	133.9 ± 14.0	132.4 ± 13.3	0.046
DBP (mmHg)	80.4 ± 10.6	81.2 ± 11.4	80.0 ± 10.2	0.050
tHcy (μmol/L)	14.63 ± 9.06	21.57 ± 13.35	11.37 ± 1.89	<0.001
FPG (mmol/L)	5.42 ± 1.54	5.29 ± 1.35	5.49 ± 1.62	0.024
TC (mmol/L)	5.25 ± 1.64	5.20 ± 2.49	5.27 ± 1.02	0.414
TG (mmol/L)	1.95 ± 1.39	2.01 ± 1.34	1.92 ± 1.41	0.254
LDL-C (mmol/L)	3.19 ± 0.87	3.14 ± 0.89	3.21 ± 0.87	0.133
Uric acid (μmol/L)	380 ± 97	420 ± 103	362 ± 89	<0.001
Creatinine (μmol/L)	83 ± 44	98 ± 67	76 ± 25	<0.001
Sedentary time <3h/d (n (%))	667 (45.5)	211 (45.0)	456 (45.8)	0.776
History of disease (n (%))				
Coronary heart disease	178 (12.2)	72 (15.4)	106 (10.6)	0.010
Cerebral hemorrhage	15 (1.0)	11 (2.3)	4 (0.4)	<0.001
Cerebral thrombosis	61 (4.2)	34 (7.2)	27 (2.7)	<0.001
Diabetes	240 (16.4)	82 (17.5)	158 (15.9)	0.434
Kidney disease	57 (3.9)	34 (7.2)	23 (2.3)	<0.001
Medications use (n (%))				
Antihypertensive	1146 (78.2)	380 (81.0)	766 (76.9)	0.075
Antihyperglycemic	21(1.4)	8 (1.7)	13 (1.3)	0.547
Lipid-lowering	21 (1.4)	5 (1.1)	16 (1.6)	0.417

BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HHcy, hyperhomocysteinemia; LDL-C, low density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; tHcy, total homocysteine; WC, waist circumference. [#]: Values are mean ± SD or median (interquartile range) or number (percentage).

Table 2 The dietary intake of food and nutrients of the participants [#]

Dietary intake (/d)	Total (n=1465)	HHcy (n=469)	nonHHcy (n=996)	*P*
Energy (kcal)	1488 (1172, 1899)	1508 (1202-1874)	1473 (1150-1913)	0.460
Vegetable (g)	383.7 (240.3, 578.0)	377.7 (227.2, 580.0)	388.2 (252.5, 577.1)	0.510
Fruit (g)	79.2 (39.7, 139.5)	75.4 (33.2, 127.5)	81.1 (42.1, 144.6)	0.036
Protein (g)	43.2 (35.4, 53.5)	41.7 (33.9-50.9)	44.2 (36.2-54.8)	<0.001
Fat (g)	54.3 (42.2, 65.6)	52.5 (41.6-64.3)	55.1 (42.6-65.9)	0.055
Carbohydrate (g)	206.6 (179.4, 234.4)	212.6 (186.6-237.7)	204.4 (176.6-231.7)	<0.001
Cholesterol (mg)	232.7 (152.3, 325.8)	228.3 (154.5, 313.7)	236.6 (147.3, 333.6)	0.229
Fibre (g)	11.2 (8.2, 15.2)	10.6 (7.9-14.8)	11.4 (8.4-15.2)	0.021
Vitamin C (mg)	153.5 (91.2, 240.9)	138.6 (85.6-231.6)	159.5 (93.6-242.7)	0.072
Vitamin E (mg)	25.0 (19.3-31.9)	24.1 (19.0-31.5)	25.4 (19.5-32.2)	0.154
Carotenes (mg)	3.3 (1.8, 5.6)	3.1 (1.7-5.5)	3.5 (1.8-5.6)	0.228
Retinol (μg)	138.3 (78.0, 283.6)	110.9 (62.2-239.3)	152.0 (83.3-317.6)	<0.001
Lutein (mg)	9.5 (5.2, 14.8)	9.0 (5.1-14.2)	9.6 (5.3-15.0)	0.269
Folate (μg)	170.4 (109.4, 263.5)	160.0 (99.9-246.2)	176.1 (112.8-266.9)	0.009

continued on next page

Table 2 *(continued)*

	Total	HHcy (n=469)	non-HHcy (n=996)	*P*
Vitamin B_6 (mg)	0.3 (0.2, 0.5)	0.3 (0.2-0.5)	0.3 (0.2-0.5)	0.233
Vitamin B_{12} (μg)	2.7 (1.6, 4.6)	2.3 (1.4-4.0)	3.0 (1.8-4.9)	<0.001
Saturated fatty acid (g)	10.6 (8.1-13.2)	10.2 (7.8-12.9)	10.8 (8.3-13.3)	0.016
Monounsaturated fatty acid (g)	12.7 (9.8-16.3)	12.4 (9.7-16.1)	12.9 (9.9-16.3)	0.111
Polyunsaturated fatty acid (g)	20.0 (14.9-26.4)	19.7 (14.9-26.0)	20.2 (15.0-26.7)	0.292
Selenium (μg)	31.3 (23.2, 41.9)	28.6 (21.0-38.8)	32.5 (24.5-43.7)	<0.001
Magnesium (mg)	216.1 (173.3- 269.2)	205.8 (167.7-261.5)	221.0 (176.3-271.6)	0.011
Zinc (mg)	7.0 (6.0- 8.3)	6.8 (5.9-7.9)	7.1 (6.1-8.5)	<0.001
Iron (mg)	16.6 (12.0- 23.4)	16.5 (11.8-23.1)	16.6 (12.1-23.6)	0.295
Supplement use (n (%))	302 (20.6)	80 (17.1)	222 (22.3)	0.021

[#]: Values are median (interquartile range) or number (percentage). HHcy, hyperhomocysteinemia.

Table 3 β (95% CIs) and ORs (95% CIs) of dietary intakes of antioxidant vitamins and homocysteine levels among the middle-aged and older hypertensive participants

	tHcy	Q1	Q2	Q3	Q4	$P_{\text{for trend}}$
Vitamin C (mg/d)		< 91.2	91.2-153.5	153.6-240.9	≥ 240.9	
Cases (%)		132 (36.1)	122 (33.3)	104 (28.4)	111 (30.2)	
Crude Model	-0.037 (-0.063, -0.011)	Ref.	0.89 (0.65, 1.20)	0.70 (0.52, 0.96)	0.77 (0.56, 1.05)	0.071
Model I	-0.031 (-0.055, -0.006)	Ref.	0.92 (0.66, 1.27)	0.64 (0.46, 0.90)	0.55 (0.34, 0.89)	0.048
Model II	-0.050 (-0.084, -0.016)	Ref.	0.82 (0.57, 1.16)	0.49 (0.33, 0.74)	0.40 (0.22, 0.74)	0.001
Vitamin E (mg/d)		< 19.3	19.3-25.0	25.1-31.9	≥ 31.9	
Cases (%)		122 (33.6)	126 (34.2)	113 (30.9)	108 (29.3)	
Crude Model	-0.035 (-0.084, 0.014)	Ref.	1.03 (0.76, 1.40)	0.88 (0.65, 1.20)	0.82 (0.60, 1.12)	0.139
Model I	-0.007 (-0.053, 0.039)	Ref.	1.10 (0.79, 1.53)	0.96 (0.69, 1.34)	0.95 (0.68, 1.33)	0.618
Model II	-0.009 (-0.055, 0.036)	Ref.	1.12 (0.80, 1.57)	0.95 (0.68, 1.33)	0.91 (0.65, 1.28)	0.412
Carotenes (mg/d)		< 1.78	1.78-3.30	3.31-5.61	≥ 5.61	
Cases (%)		118 (32.7)	136 (37.0)	99 (26.8)	116 (31.6)	
Crude Model	-0.024 (-0.045, -0.003)	Ref.	1.21 (0.89, 1.64)	0.76 (0.55, 1.04)	0.95 (0.70, 1.30)	0.315

continued on next page

Table 3 (*continued*)

	tHcy	Q1	Q2	Q3	Q4	$P_{\text{for trend}}$
Model I	-0.022 (-0.042, -0.003)	Ref.	1.22 (0.88, 1.69)	0.72 (0.51, 1.01)	0.93 (0.67, 1.30)	0.254
Model II	-0.022 (-0.052, 0.009)	Ref.	1.18 (0.84, 1.68)	0.68 (0.46, 1.02)	0.76 (0.43, 1.35)	0.159
Retinol (μg/d)		< 78.0	78.0-138.3	138.4-283.6	≥ 283.6	
Cases (%)		147 (40.2)	133 (36.3)	91 (24.9)	98 (26.7)	
Crude Model	-0.030 (-0.046, -0.015)	Ref.	0.85 (0.63, 1.15)	0.49 (0.36, 0.68)	0.54 (0.40, 0.74)	0.001
Model I	-0.027 (-0.041, -0.012)	Ref.	0.84 (0.61, 1.16)	0.50 (0.35, 0.70)	0.55 (0.40, 0.77)	0.003
Model II	-0.021 (-0.041, -0.002)	Ref.	0.90 (0.65, 1.25)	0.61 (0.42, 0.86)	0.86 (0.56, 1.32)	0.951
Lutein (mg/d)		< 5.22	5.22-9.48	9.49-14.82	≥ 14.82	
Cases (%)		123 (33.7)	120 (32.8)	114 (31.1)	112 (30.4)	
Crude Model	-0.028 (-0.053, -0.004)	Ref.	0.96 (0.71, 1.31)	0.89 (0.65, 1.21)	0.86 (0.63, 1.17)	0.311
Model I	-0.024 (-0.046, -0.001)	Ref.	0.98 (0.71, 1.37)	0.84 (0.61, 1.17)	0.87 (0.62, 1.22)	0.331
Model II	-0.025(-0.063, 0.013)	Ref.	0.92 (0.65, 1.31)	0.76 (0.51, 1.14)	0.66 (0.37, 1.19)	0.138

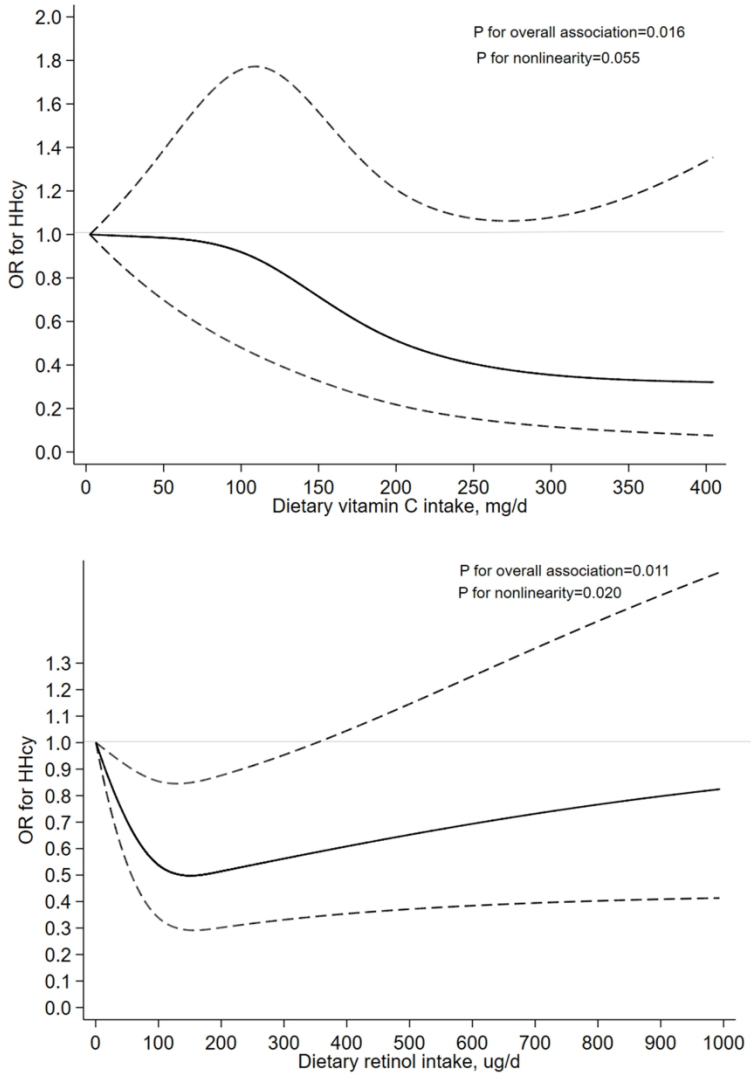
Model I: Adjusted for age, sex ('male' as the reference) BMI.

Model II: Adjusted for variables in Model I and further adjusted for sedentary time ('< 3 h/d' as the reference), the intakes of folate, vitamin B6 and vitamin B12, supplement use ('no' as the reference) and the history of cardiovascular events ('no' as the reference).

Figure Legends:

Fig. 1 Restricted cubic spline analyses illustrating the shapes of multivariable association between dietary vitamin C intake (A), or dietary retinol intake (B) and HHcy. Adjusted for age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin B_6 and vitamin B_{12}, the history of cardiovascular events ('no' as the reference).

Fig. 2 ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to vitamin C, retinol intake in subgroups, stratified by sex (A), BMI (B). Adjusted for age, sex (except sex subgroup), BMI (except BMI subgroup), sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin B6 and vitamin B12, the history of cardiovascular events ('no' as the reference).



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187x287mm (300 x 300 DPI)

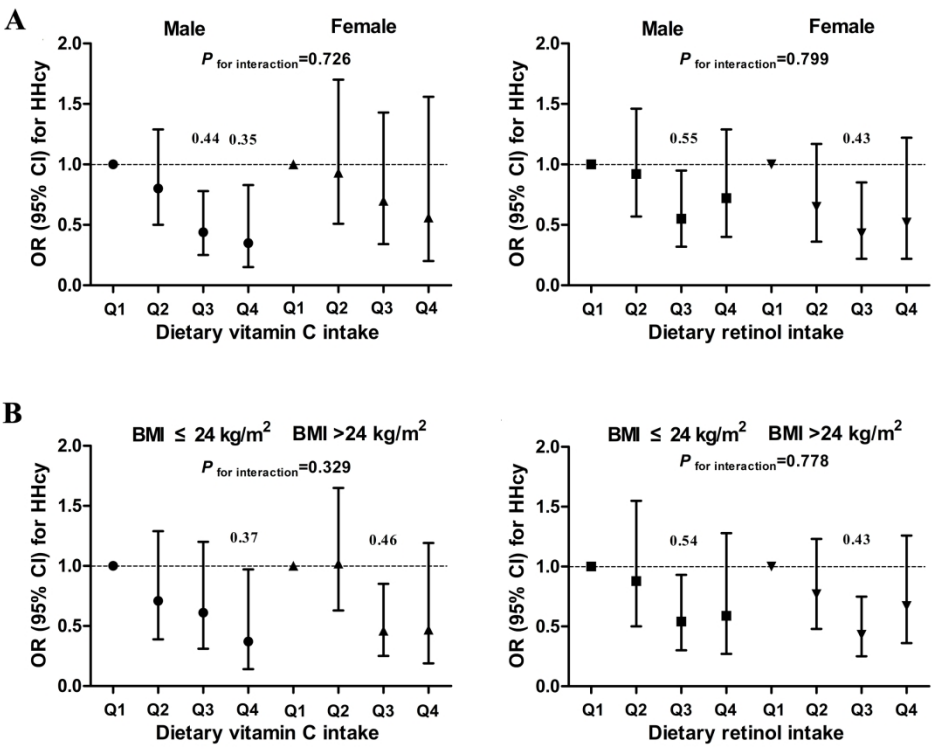


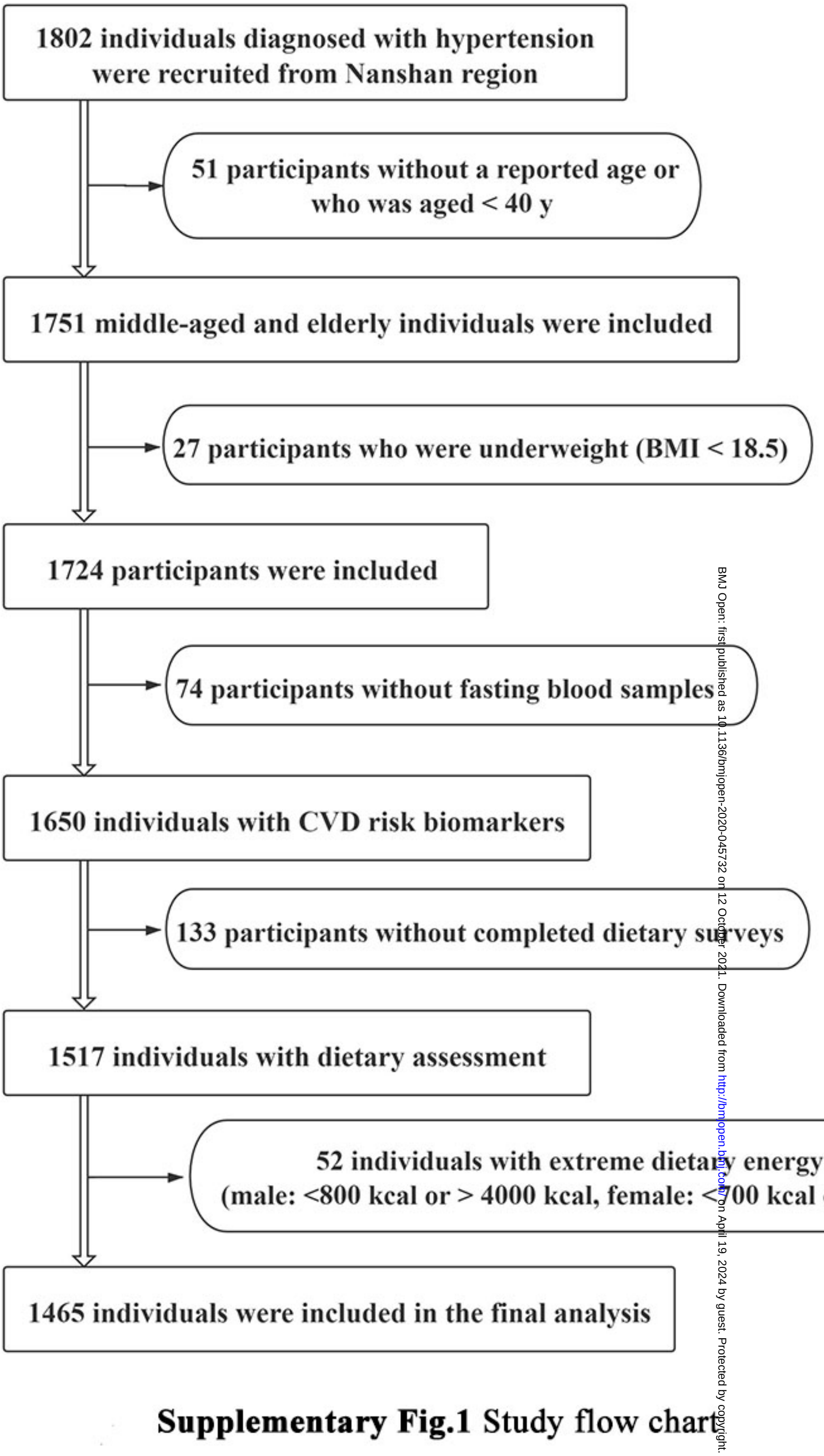
Fig. 2 ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to vitamin C, retinol intake in subgroups, stratified by sex (A), BMI (B). Adjusted for age, sex (except sex subgroup), BMI (except BMI subgroup), sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin B6 and vitamin B12, the history of cardiovascular events ('no' as the reference).

127x96mm (600 x 600 DPI)

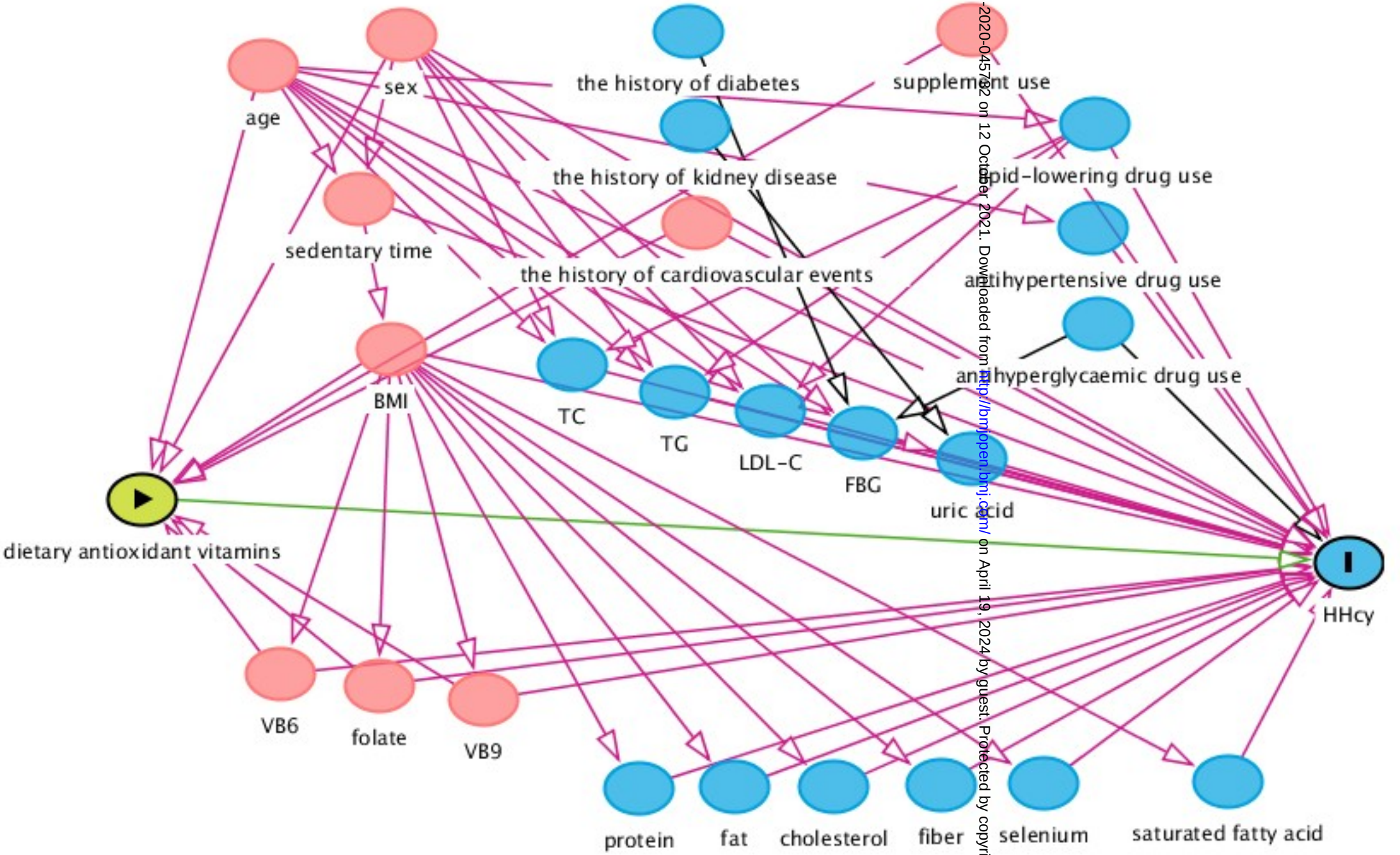
Supplementary Table 1 Sensitivity analyses of ORs (95% CIs) of antioxidant vitamins intake for hyperhomocysteinemia prevalence in multivariable-adjusted model among the patients with hypertension [#]

	Q1	Q2	Q3	Q4	$P_{\text{for trend}}$
Without cardiovascular events (n=1245)					
Vitamin C	1.00 (Ref.)	0.92 (0.63, 1.35)	0.54 (0.35, 0.84)	0.41 (0.21, 0.80)	0.003
Vitamin E	1.00 (Ref.)	1.14 (0.79, 1.64)	0.95 (0.66, 1.39)	0.90 (0.62, 1.31)	0.425
Carotenes	1.00 (Ref.)	1.25 (0.85,1.84	0.67 (0.43, 1.04)	0.77 (0.41, 1.44)	0.178
Retinol	1.00 (Ref.)	1.05 (0.73, 1.50)	0.61 (0.43, 0.94)	0.78 (0.50, 1.34)	0.836
Lutein	1.00 (Ref.)	1.04 (0.71, 1.53)	0.74 (0.48, 1.14)	0.63 (0.33, 1.20)	0.104
Without tHcy-lowering drug-using (n=1360)					
Vitamin C	1.00 (Ref.)	0.85 (0.58, 1.24)	0.49 (0.31, 0.78)	0.44 (0.22, 0.86)	0.007
Vitamin E	1.00 (Ref.)	1.07 (0.74, 1.55)	0.98 (0.68, 1.42)	0.95 (0.66, 1.37)	0.685
Carotenes	1.00 (Ref.)	1.27 (0.87, 1.85)	0.69 (0.44, 1.07)	0.67 (0.35, 1.28)	0.090
Retinol	1.00 (Ref.)	0.84 (0.59, 1.20)	0.56 (0.38, 0.84)	0.85 (0.54, 1.35)	0.837
Lutein	1.00 (Ref.)	0.88 (0.60, 1.29)	0.74 (0.47, 1.15)	0.60 (0.31, 1.16)	0.115

[#]: Adjusted for age, sex, BMI, sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin B_6 and vitamin B_{12}.



Supplementary Fig.1 Study flow chart



Supplementary Figure 2 Directed acyclic graph drawn in the DAGitty programme to identify the minimum set of confounders (depicted in red) to enter the adjusted model to examine the association between dietary intakes of antioxidant vitamins and HHcy

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	# 1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	# 2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	# 3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	# 4
Methods			
Study design	4	Present key elements of study design early in the paper	# 4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	# 4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	# 4-5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	# 5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	# 5-7
Bias	9	Describe any efforts to address potential sources of bias	# *7*
Study size	10	Explain how the study size was arrived at	# 4-5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	# 6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	# 7-8
		(*b*) Describe any methods used to examine subgroups and interactions	# 7-8
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	# 7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	# 8
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	# 5
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	# 8
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	# 8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	# 8-9
		(*b*) Report category boundaries when continuous variables were categorized	# 8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	# 9
Discussion			
Key results	18	Summarise key results with reference to study objectives	# 9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	# 12-13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	# 10-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	# 13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	# 13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association between dietary antioxidant vitamins intake and homocysteine levels in middle-aged and older adults with hypertension: a cross-sectional study

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Abstract

Objectives: Plasma total homocysteine (tHcy) has been implicated in the development of cardiovascular disease. This study aimed to assess the relationship of dietary antioxidant vitamins intake with tHcy levels in middle-aged and older adults with hypertension.

Design: A cross-sectional study.

Setting: The survey was conducted in the Nanshan district of Shenzhen.

Participants: A total of 1465 middle-aged and older adults with hypertension were included between July and September of 2013.

Measurements: Hyperhomocysteinemia (HHcy) was defined as tHcy ≥ 15 μmol/L. Some dietary antioxidant vitamins (vitamin C (VC) and vitamin E (VE), carotenes, retinol, lutein) intake was estimated using the food frequency questionnaire. Socio-demographic and potential covariates were evaluated through questionnaires, anthropometric measurements and blood tests. The association between dietary intakes of antioxidant vitamins and tHcy concentration were evaluated by multiple linear regression analyses after napierian logarithm -transformed. Multiple logistic regression models were further used to determine odds ratios (ORs) and 95% confidence intervals (CIs).

Results: The β (95% CIs) of VC intake and tHcy was -0.050 (-0.084, -0.016). Compared with the lowest quartile in the fully adjusted model, the ORs (95% CIs) for HHcy levels across quartiles of dietary VC intake were 0.82 (0.57, 1.16), 0.49 (0.33, 0.74) and 0.40 (0.22, 0.74) (*P* for trend=0.001). The β (95% CIs) of retinol intake and tHcy was -0.021 (-0.041, -0.002), and the ORs (95% CIs) in the third quartile of retinol intake was 0.61 (0.42, 0.86), while the effect for the highest quartile was not significant (*P* for trend=0.951). No significant association was observed between dietary VE, carotenes and lutein intake and HHcy.

Conclusions: A linear inverse association between dietary VC intake and HHcy prevalence, and an L-shaped association between dietary retinol intake and HHcy prevalence were found in Chinese middle-aged and older adults with hypertension.

Strengths and limitations of this study:

1. This study focused on the risk of hyperhomocysteinemia among middle-aged and older adults with hypertension.
2. A linear inverse association between dietary VC intake and HHcy prevalence and the threshold effect of retinol on HHcy was reported in this study.
3. Based on a cross-sectional study design, we could only draw a conclusion about correlation, not causation.
4. Although some confounding factors were included in the analysis, other potential confounders may exist.

Introduction

Increasing evidence has shown that elevated total homocysteine (tHcy) levels are associated with atherosclerotic cardiovascular diseases, stroke, peripheral arterial occlusive disease, and venous thrombosis (1, 2). Hyperhomocysteinemia (HHcy) (tHcy $\geq$ 15 μmol/L) (3), the result of a disturbed methionine metabolism, may lead to an enhancement of the adverse effects of risk factors like hypertension on human health (4). Hypertension is a major risk factor for cardiovascular and chronic kidney disease and a major cause of the global burden of disease and mortality (5, 6). It was estimated that the prevalence of hypertension in China in 2015 was 23.2%, and about 245 million Chinese adults had hypertension (7). Notably, the incidence of hypertension with hyperhomocysteinemia, or 'H-type hypertension', is significantly higher compared with other countries, representing 75% of Chinese patients with hypertension (8). Thus, the nutritional treatment focus on the attempt to reduce cardiovascular risk by reducing tHcy is important, particularly among patients with hypertension (9).

Increasing age, male sex, genetic factors, consumption of alcohol or tobacco, kidney function and physical activity are some of the factors associated with tHcy levels (10). Epidemiological studies and clinical trials have indicated that folate, vitamin B_{12} and vitamin B_6 status, well-known predictors of tHcy, are important for tHcy metabolism. The latest meta-analysis demonstrated that a lower risk of stroke and overall cardiovascular disease (CVD) with folic acid supplementation, which may partly

contribute to the decrease of tHcy levels (11).

Folic acid and vitamin B_{12}, which are involved in remethylation of homocysteine to methionine by methionine synthase (MS), and vitamin B_6, which acts as a cofactor in the transsulfuration of homocysteine to cysteine by cystathionine β-synthase (CBS) (12). Folate, a key factor of tHcy metabolism, is very sensitive to free radicals (13-15). In addition, MS and CBS were strongly influenced by oxidative stress, which may be associated with dietary antioxidant vitamins and Hcy levels (16,17). We hypothesized that antioxidant nutrients may regulate tHcy metabolism by influencing methionine-homocysteine cycle. Plasma levels or dietary intake of vitamin C (VC), vitamin E (VE) or β-carotene was inversely associated with tHcy levels, however, the findings were not consistent (18-20). Of note, the association has never been investigated among the hypertensive population.

Therefore, this large population-based study aimed to determine the association between the dietary intake of VC, VE, carotenes, retinol and lutein and the prevalence of HHcy in middle-aged and older men and women with hypertension.

Methods

Study design and population

This study consecutively recruited individuals with hypertension from 60 community health service centres (CHSCs) in the Nanshan district of Shenzhen from July to September of 2013 using a three-stage random sampling method. In the first stage, 8 sub-districts were selected in the Nanshan district; in the second stage, 6 to 8 communities were selected from each sub-district using a simple random selection procedure; and in the third stage, individuals with hypertension were selected from each community using isometric random sampling. All subjects were of Chinese ethnicity and had lived in the Nanshan district of Shenzhen for over six months. The individuals were invited to visit the CHSCs, where the researcher-administered questionnaire (including the validated food frequency questionnaire (FFQ)) was conducted, the anthropometric measurements were recorded, and fasting blood samples were collected.

We collected the data of 1802 participants, and excluded 51 participants whose age was not reported or who were aged ≤ 40 years and 27 underweight participants (body

mass index (BMI) < 18.5 kg/m^2), as well as participants for whom fasting blood samples (n = 74) and complete dietary surveys (n = 133) were not available. We also excluded participants with an extreme dietary energy intake (male: < 800 kcal or > 4000 kcal, female: < 700 kcal or > 3500 kcal) (n = 52). Finally, 1465 participants were included in the analysis **(Supplementary Figure 1)**.

Patient and public involvement

The development of standardised form is in response to the public health need of preventing stroke among hypertension population. Patients and the public were not involved in the design of the study. The results of our study will be disseminated through open access publications.

Dietary assessment

The researcher-administered FFQ consisted of 92 food items, which were assembled into 7 food groups: cereals, vegetables, fruits, beans, animal foods, nuts and beverages. The FFQ was based on the national FFQ used in the 2010-2012 China National Nutrition and Health Survey according to the Chinese Nutrition and Health Surveillance in 2010-2012 (21). Participants were asked to recall the consumption of each item during the past year, including the type of food, frequency and amount. Food weight maps were available for participants to estimate their portion size. Primary data obtained from FFQs were doubly entered into the EpiData 3.0 software by two trained technicians to verify accuracy. Dietary energy and other nutrients were calculated based on the Chinese Food Composition Database (22, 23). Dietary intake of VC, VE, carotenes (consist of α-carotene, β-carotene and β-cryptoxanthin), and retinol were calculated based on the Chinese Food Composition Table 2002 (22). We report carotenes and retinol separately, because we could not get the accurate calculation for vitamin A, as the data for carotenes were combined with different carotene, rather than separate one. Dietary intake of lutein was calculated based on the food composition table of vegetables, fruits, eggs and nuts that contain large amounts of lutein, according to Chinese Dietary Reference Intakes 2013 (24). The intake of all dietary nutrients and carotenoids was adjusted for energy using the residual method (25). The second FFQ was conducted 3 weeks after the completion of the first FFQ among 108 participants.

The intra-class correlation coefficients of two administrations of FFQ for nutrients of VC, VE, carotenes, retinol, lutein were 0.395, 0.477, 0.355, 0.551 and 0.350, and were all statistically significant.

Assessment of other covariates

Participants' socio-demographic characteristics (e.g., age and sex), lifestyle factors (e.g., sedentary time), history of chronic diseases, and medication and supplement use status were collected. Sedentary time consisted of time spent watching TV and sitting, which were combined into one variable with 2 categories, < 3 h/d and ≥ 3 h/d, based on a median sedentary time of 3 h/d. The history of cardiovascular events including coronary heart disease, cerebral haemorrhage, cerebral thrombosis, and the history of diabetes and kidney disease were recorded for each participant. The prescription use status was classified into 2 groups (yes or no) corresponding to whether the participant was taking any type or quantity of drugs, including antihypertensive drugs, antihyperglycaemic drugs, lipid-lowering drugs or tHcy-lowering drugs. Supplement use consisted of vitamin A, retinol, B vitamins, VC, VE and folic acid was included.

Anthropometric measurements

Height and weight and waist circumference (WC) were measured by specialists. BMI was calculated as weight (kg) / height (m^2). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured from the right arm of participants in a seated position after a sufficient rest period using a mercury sphygmomanometer in the morning. Blood pressure was measured manually and recorded as the average of three measurements.

Laboratory tests and outcomes

Morning fasting elbow vein blood samples were required to fast overnight (at least 10 hours) were collected from the participants at the CHSCs and transported under refrigerated conditions to a clinical laboratory of the Nanshan Centre for Chronic Disease Control within 2 hours. The blood specimens were collected in a 5-ml EDTA vacuum tube. Blood samples were collected through deposition and centrifugation for ten minutes at 3000 r/min at room temperature. The concentrations of plasma tHcy, fasting plasma glucose (FPG), total cholesterol (TC), triglyceride (TG), low-density

lipoprotein cholesterol (LDL-C), uric acid and creatinine were assessed on the day of blood collection using enzymatic methods via an auto-analyser (HITACHI 7080). The inter-day quality control assessments met the standard during the analysis. HHcy was defined as plasma tHcy concentration ≥ 15 μmol/L.

Statistical analysis

Demographic characteristics were described by means ± SDs for normally distributed data, medians (interquartile ranges, IQRs) for non-normally distributed data and numbers (percentages) for categorical data. The differences between males and females were compared using the *t* test for normally distributed variables, the Kruskal-Wallis rank test for non-normally distributed variables, and the chi-square test for categorical variables.

Both dietary intakes of antioxidant vitamins and tHcy concentration were napierian logarithm (ln)-transformed to improve normality before analyses and categorised into quartiles. The associations between dietary antioxidant vitamins intake and the prevalence of HHcy were analyzed using multiple logistic regression models, with the lowest quartile as the reference category. Confounding variables were selected based on the minimal sufficient adjustment recommended by the Directed Acyclic Graph, created in the online software Dagitty 3.0 (**Supplementary Figure 2**). The selected potential confounders included age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as the reference), the intakes of folate, vitamin B_6 and vitamin B_{12}, supplement use ('no' as the reference) and the history of cardiovascular events ('no' as the reference). Linear trends were tested by creating a continuous variable for dietary antioxidant vitamins intake using the median value for each quartile. The sensitive analyses between dietary antioxidant vitamins intake and HHcy prevalence were applied among the population who had never suffered cardiovascular events, or among the population who never use the tHcy-lowering drug.

To further explore the nonlinearity of the relationship between dietary antioxidant vitamins intake and high tHcy, we used restricted cubic spline with 4 knots at the 5^{th}, 35^{th}, 65^{th} and 95^{th} percentiles of dietary antioxidant vitamins intake.

To evaluate the modification effect by some potential prevalence factors of HHcy,

including sex (male or female) and BMI (<24 or ≥24 kg/m^2), stratified analyses were conducted by these potential factors and estimated *P* values for interaction terms.

All data were analyzed using the R Foundation version 3.1.2 and Empower X&Y Solutions Inc. Statistical significance was considered when $P < 0.05$ (two-sided).

Results

In this cross-sectional study, 1465 participants (male: 729, female: 736) were included in the analysis, and the mean ± SD of age and BMI was 62.0 ± 10.7 years, and 24.9 ± 3.5 kg/m^2. The tHcy levels of the participants was 14.63 ± 9.06 μmol/L, and the number (percentage) of HHcy was 469 (32.0%). The participants with HHcy were older and more male, and have higher WC, SBP, tHcy, uric acid, creatinine, and higher percentages of history of cardiovascular events (including coronary heart disease, cerebral hemorrhage, cerebral thrombosis) and kidney disease, and have lower FPG level (all $P < 0.05$) (**Table 1**).

The dietary intakes of nutrients were shown in **Table 2**. The median (IQR) of dietary antioxidant vitamins intake were as follows: VC 153.5 (91.2, 240.9) mg/d, VE 25.0 (19.3, 31.9) mg/d, carotenes 3.3 (1.8, 5.6) mg/d, retinol 138.3 (78.0, 283.6) μg/d, and lutein 9.5 (5.2, 14.8) mg/d. Among the participants with HHcy, the intake of carbohydrate was higher, and the intake of fruit, protein, fibre, retinol, folate, vitamin B_{12}, saturated fatty acid, selenium, magnesium, zinc was lower, and the percentage of supplement use was lower (all $P < 0.05$).

The association between dietary antioxidant vitamins intake and tHcy levels are shown in **Table 3**. The inverse association between VC intake and tHcy concentration after ln-transformed in the fully adjusted model, and the β (95% CIs) was -0.050 (-0.084, -0.016), which was consistent with the logistic regression as categorical variables. In Model II, the significant association was found in the third and highest quartile of VC intake, and the ORs (95% CIs) were 0.49 (0.33, 0.74) and 0.40 (0.22, 0.74) ($P_{\text{for trend}} = 0.001$). The retinol intake was also inversely associated with tHcy, as the β (95% CIs) was -0.021 (-0.041, -0.002). After adjusting for age, sex and BMI, the ORs (95% CIs) for HHcy prevalence across quartiles of retinol intake were 1.00, 0.84 (0.61, 1.16), 0.50 (0.35, 0.70), and 0.55 (0.40, 0.77) ($P_{\text{for trend}} = 0.003$). However, in

Model II, the significant association was only found in the third quartile of retinol intake, and the ORs (95% CIs) was 0.61 (0.42, 0.86) ($P_{\text{for trend}}$ =0.951). The non-significant association between dietary intake of VE, carotenes and lutein and HHcy prevalence was found. The results were similar in the sensitivity analyses that excluded participants with cardiovascular events or who use the tHcy-lowering drug (see **Supplementary Table 1**).

After fully adjusting for the potential confounders, the association between dietary antioxidant vitamins intake and the prevalence of HHcy were shown in **Figure.1**. From the cubic splines, we noted that the linear inverse trend of VC intake and HHcy prevalence (*P* for overall association=0.016, *P* for nonlinearity=0.055) and the L-shaped relationship of retinol intake and HHcy prevalence (*P* for overall association=0.011, *P* for nonlinearity=0.020), which were consistent with the results of logistic regression analyses. The threshold analysis showed that the ORs (95% CIs) of HHcy was 0.995 (0.991, 0.995) (*P*=0.005) when retinol intake was lower than 147.2 μg/d and 1.000 (1.000, 1.001) (*P*=0.094) when retinol intake was more than 147.2 μg/d, and the *P* value of log-likelihood ratio was 0.003. The non-association between carotenes, lutein and VE and HHcy was not shown.

In stratified analyses, the association between dietary VC and retinol intake and HHcy prevalence were not significantly modified by sex (male or female), BMI (<24 or ≥24 kg/m^2) (all $P_{\text{for interaction}}$ were >0.05) (**Figure 2**). Similar results of stratified analyses of carotenes, lutein and VE were not shown.

Discussion

In this community-based cross-sectional study, we observed some of the antioxidant vitamins intakes were significantly correlated with the prevalence of HHcy. After adjusting potential confounders, a linear inverse association between VC intake and HHcy prevalence, and an L-shaped relationship between retinol intake and HHcy prevalence were found, which were not modified by sex or BMI. However, the non-significant effect of VE, carotenes and lutein on HHcy was detected.

Numerous studies have suggested that HHcy may be a modifiable risk factor for CVD, especially for stroke. In the past decades, there were many clinical trials aimed

to show the effect of folate and vitamins B_{12} and B_6 supplement on lowering the levels of tHcy, however, the negative results were found in most studies (26). The potential reason may be correlated with the harmful effect of unmetabolized excessive folate or the cyanide and thiocyanate from excessive cyanocobalamin. In China, where folate fortification has not been implemented, folic acid significantly reduced the risk of stroke was observed from the China Stroke Primary Prevention Trial (CSPPT) (27). Thus, appropriate B vitamins therapy is of great importance for lowering tHcy levels in stroke prevention.

In the past decades, several researches reported the potential association between plasma levels or intake of different antioxidant vitamins and tHcy levels (18, 19, 28-30). In 1999, Brude IR *et al.* observed the inverse association between plasma tHcy concentration and dietary intake of vegetables, vitamin C and β-carotene from 41 participants (28). In addition, dietary intake of retinol equivalents, β-carotene and VC were inversely correlated with plasma tHcy levels, after adjustment for dietary B-vitamins, but not after additional adjustment for plasma folate and vitamin B_{12} (19). What's more, the study focused on the effect of antioxidant vitamins on the plasma tHcy levels in a free-living elderly population found that plasma VC, rather than the intake and supplementation of VC, showed a negative association with tHcy in simple regression analysis, and also found that the plasma levels, as well as the intake and supplementation of vitamin E, and β-carotene were not associated with tHcy (18). Similarly, the cross-sectional NHANES 1999–2002 study found that dietary VC and VE intake were associated with a lower prevalence of elevated blood tHcy concentration, whereas no association between dietary carotenes intake and tHcy was detected (29). In addition, Rajesh Ullegaddi et al. found that significant reductions in plasma homocysteine in the group with antioxidant treatment (vitamins C and E) combination with B-vitamin, compared with the group with B-vitamin alone (30), which support the importance of antioxidant vitamins on tHcy metabolism.

Consistent with our findings, these studies have a common conclusion that VC intake was inversely correlated with tHcy levels. Given the report of an interaction of VC and folate (31), Magana AA *et al.* found the underlying molecular mechanisms that VC

activates the folate-mediated one-carbon cycle in C2C12 myoblasts (32). Thus, VC has been explored as an attractive factor to increase circulating levels of folic acid and to reduce Hcy levels.

We found an L-shaped association between dietary retinol intake and high tHcy prevalence, which meant that if the retinol intake was low, the risk of HHcy was decreased as retinol intake increased, but the risk was not changed when retinol intake reached certain level, which was more than 147.2 μg/d among the participants in this study. The dietary intake of retinol equivalents was inversely correlated with plasma tHcy levels after adjustment for dietary B-vitamins (19). Retinol, a preformed vitamin A, plays an important role in vision, cellular differentiation, and proliferation, as well as the immune system regulation. In addition, there is increasing evidence indicates that retinol seems to inhibit thrombosis (33) and inflammation effects (34), which indicates retinol is emerging as a factor of interest to CVD. Brazionis L *et al.* reported that plasma retinol was a novel marker for CVD mortality in Australian adults, with an inverse association between plasma retinol in the middle tertile and 5-year CVD mortality (35). Similarly, a strong association between low retinol and the risk of sudden cardiac death was examined (36). In addition, a nested case-control study showed a significant inverse association between plasma retinol and the risk of first stroke among Chinese hypertensive adults from the CSPPT (37), which may due to relatively low baseline retinol concentrations (median: 67.5 μg/dL). Besides, the interaction of retinol and tHcy on CVD risk was also reported. Yu Y *et al.* (37) showed the inverse effect between plasma retinol and first stroke was stronger among the participants whose tHcy < 10 μmol/L than whose tHcy ≥ 10 μmol/L. Olsen T *et al.* (38) found that the plasma tHcy was associated with acute myocardial infarction only in the upper Vit-A tertile, and the potential mechanisms may include inflammation and lipid metabolism, which may be partly interpreted with the high intake of retinol (1576 μg RAE/d).

In addition, the dietary source of retinol may contribute to the non-significant effect of retinol in the highest quartile in the fully adjusted model, as it is present in animal-based foods, particularly in liver and whole milk. In this study, we found the TG level was gradually increased with the increase of retinol (data not shown). High retinol

intake may alter lipid metabolism by increasing TG level, which may impact the tHcy metabolism (39).

At present, a few studies demonstrate the complicated relationship between retinol, tHcy and CVD risk, but the underlying mechanism has not yet been clarified (40). The antioxidant activity of retinol may be a plausible mechanism that links the effect of retinol on tHcy levels, as retinol is essential for the maintenance of immune function and antioxidant defence (41, 42).

However, we found no relationship between HHcy prevalence and dietary intake of VE. The aforementioned study has found significant inverse associations between plasma tHcy and dietary intake of VE, but the effect was not significant after adjusting B-vitamins intake, which was consistent with our finding (19). On the contrary, dietary VE (α-tocopherol) intake was associated with a lower risk of elevated blood tHcy concentration among US adults (29).

Epidemiological studies have reported the positive role of carotenoids on human health. In this study, we focused on the effect of carotenes (β-carotene, α-carotene and β-cryptoxanthin) and lutein on tHcy, however, the negative results were found. Many of the aforementioned studies reported the protective effect of β-carotene by lowering tHcy concentration (19, 28), but the risk of tHcy > 13 μmol/L was associated with the total carotene intake from diet plus supplement use, rather than the only intake from diet (29). The negative finding of lutein cannot be compared because of a lack of previously reported data. Thus, more prospective cohort studies and randomized double trials are warranted to indicate the relationship of antioxidant vitamins with HHcy risk.

The strength of this study is the face-to-face researcher-administered FFQ survey. First, to our knowledge, this is the first study to demonstrate an association of dietary intake of antioxidant vitamins with HHcy prevalence that was conducted in both a male and female hypertensive population. Then, the researcher-administered face-to-face interview was considered by validated FFQ, which was designed to evaluate dietary intake and gave full consideration to eating habits and food nutrient composition in the Chinese population.

Our study has several limitations. First, based on a cross-sectional study design, we

cannot draw a conclusion about causality. The strong effects observed in this study may be enhanced because of selection effects, because hypertensive individuals may have made changes to their diet in response to diagnosis. Second, we adjusted the dietary intake of folate, B_{12}, B_6, rather than plasma concentrations, which may could not eliminate confounding effect. Just like Konstantinova SV (19) reported, the inverse correlation of dietary retinol intake and plasma tHcy disappeared after adjustment for plasma folate and vitamin B_{12}. Then, we investigated the participants whether they took dietary supplements, while the detailed doses were not recorded. Therefore, we are not able to eliminate the possible association between supplement use and HHcy prevalence. Nevertheless, the study reported that there was some potential benefit from the antioxidant supplementation on plasma tHcy concentration (29), which could stabilize the positive results in this study. In addition, although some confounding factors were included in the analysis, other potential confounders may exist. For instance, the influence of smoking and drinking on HHcy was not assessed because of the lack of information about the status of smoking and drinking.

In conclusion, we found dietary intake of VC and retinol was inversely associated with HHcy prevalence in middle-aged and older adults with hypertension after adjusting the potential confounders. Our findings have provided suggestive evidence of an inverse relationship between certain antioxidant intake and HHcy, which should be the impetus for longitudinal and random control trails to verify the relationship and direction and to elucidate the underlying mechanisms in the future.

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Contributors

The authors' responsibilities were as follows: H.L. conceived and designed the study and critically revised the manuscript. P.X. and G.Q. analyzed the data and wrote the paper. Z.J. participated in the laboratory assay. M.J. and Z.D. collected the data and revised the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

No, there are no competing interests for any author.

Ethics approval

The survey protocol was approved by the Ethics Committee of the Shenzhen Nanshan Centre for Chronic Disease Control (ID: ll20190003), and all participants provided written informed consent before enrolment.

Data availability statement

No data are available.

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Table 1 Basic characteristics of the participants [#]

	Total (n=1465)	HHcy (n=469)	non-HHcy (n=996)	*P*
Age (years)	62.0 ± 10.7	64.4 ± 11.4	60.9 ± 10.2	<0.001
Gender (Male, n (%))	729 (49.8)	339 (72.3)	390 (39.2)	<0.001
BMI (kg/m^2)	24.9 ± 3.5	25.0 ± 3.4	24.8 ± 3.6	0.252
WC (cm)	87.3 ± 9.5	88.7 ± 10.0	86.7 ± 9.2	<0.001
SBP (mmHg)	132.9 ± 13.5	133.9 ± 14.0	132.4 ± 13.3	0.046
DBP (mmHg)	80.4 ± 10.6	81.2 ± 11.4	80.0 ± 10.2	0.050
tHcy (μmol/L)	14.63 ± 9.06	21.57 ± 13.35	11.37 ± 1.89	<0.001
FPG (mmol/L)	5.42 ± 1.54	5.29 ± 1.35	5.49 ± 1.62	0.024
TC (mmol/L)	5.25 ± 1.64	5.20 ± 2.49	5.27 ± 1.02	0.414
TG (mmol/L)	1.95 ± 1.39	2.01 ± 1.34	1.92 ± 1.41	0.254
LDL-C (mmol/L)	3.19 ± 0.87	3.14 ± 0.89	3.21 ± 0.87	0.133
Uric acid (μmol/L)	380 ± 97	420 ± 103	362 ± 89	<0.001
Creatinine (μmol/L)	83 ± 44	98 ± 67	76 ± 25	<0.001
Sedentary time <3h/d (n (%))	667 (45.5)	211 (45.0)	456 (45.8)	0.776
History of disease (n (%))				
Coronary heart disease	178 (12.2)	72 (15.4)	106 (10.6)	0.010
Cerebral hemorrhage	15 (1.0)	11 (2.3)	4 (0.4)	<0.001
Cerebral thrombosis	61 (4.2)	34 (7.2)	27 (2.7)	<0.001
Diabetes	240 (16.4)	82 (17.5)	158 (15.9)	0.434
Kidney disease	57 (3.9)	34 (7.2)	23 (2.3)	<0.001
Medications use (n (%))				
Antihypertensive	1146 (78.2)	380 (81.0)	766 (76.9)	0.075
Antihyperglycemic	21(1.4)	8 (1.7)	13 (1.3)	0.547
Lipid-lowering	21 (1.4)	5 (1.1)	16 (1.6)	0.417

BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HHcy, hyperhomocysteinemia; LDL-C, low density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; tHcy, total homocysteine; WC, waist circumference. [#]: Values are mean ± SD or median (interquartile range) or number (percentage).

Table 2 The dietary intake of food and nutrients of the participants [#]

Dietary intake (/d)	Total (n=1465)	HHcy (n=469)	nonHHcy (n=996)	*P*
Energy (kcal)	1488 (1172, 1899)	1508 (1202-1874)	1473 (1150-1913)	0.460
Vegetable (g)	383.7 (240.3, 578.0)	377.7 (227.2, 580.0)	388.2 (252.5, 577.1)	0.510
Fruit (g)	79.2 (39.7, 139.5)	75.4 (33.2, 127.5)	81.1 (42.1, 144.6)	0.036
Protein (g)	43.2 (35.4, 53.5)	41.7 (33.9-50.9)	44.2 (36.2-54.8)	<0.001
Fat (g)	54.3 (42.2, 65.6)	52.5 (41.6-64.3)	55.1 (42.6-65.9)	0.055
Carbohydrate (g)	206.6 (179.4, 234.4)	212.6 (186.6-237.7)	204.4 (176.6-231.7)	<0.001
Cholesterol (mg)	232.7 (152.3, 325.8)	228.3 (154.5, 313.7)	236.6 (147.3, 333.6)	0.229
Fibre (g)	11.2 (8.2, 15.2)	10.6 (7.9-14.8)	11.4 (8.4-15.2)	0.021
Vitamin C (mg)	153.5 (91.2, 240.9)	138.6 (85.6-231.6)	159.5 (93.6-242.7)	0.072
Vitamin E (mg)	25.0 (19.3-31.9)	24.1 (19.0-31.5)	25.4 (19.5-32.2)	0.154
Carotenes (mg)	3.3 (1.8, 5.6)	3.1 (1.7-5.5)	3.5 (1.8-5.6)	0.228
Retinol (μg)	138.3 (78.0, 283.6)	110.9 (62.2-239.3)	152.0 (83.3-317.6)	<0.001
Lutein (mg)	9.5 (5.2, 14.8)	9.0 (5.1-14.2)	9.6 (5.3-15.0)	0.269
Folate (μg)	170.4 (109.4, 263.5)	160.0 (99.9-246.2)	176.1 (112.8-266.9)	0.009

continued on next page

Table 2 *(continued)*

	Total	HHcy (n=469)	non-HHcy (n=996)	*P*
Vitamin B_6 (mg)	0.3 (0.2, 0.5)	0.3 (0.2-0.5)	0.3 (0.2-0.5)	0.233
Vitamin B_{12} (μg)	2.7 (1.6, 4.6)	2.3 (1.4-4.0)	3.0 (1.8-4.9)	<0.001
Saturated fatty acid (g)	10.6 (8.1-13.2)	10.2 (7.8-12.9)	10.8 (8.3-13.3)	0.016
Monounsaturated fatty acid (g)	12.7 (9.8-16.3)	12.4 (9.7-16.1)	12.9 (9.9-16.3)	0.111
Polyunsaturated fatty acid (g)	20.0 (14.9-26.4)	19.7 (14.9-26.0)	20.2 (15.0-26.7)	0.292
Selenium (μg)	31.3 (23.2, 41.9)	28.6 (21.0-38.8)	32.5 (24.5-43.7)	<0.001
Magnesium (mg)	216.1 (173.3- 269.2)	205.8 (167.7-261.5)	221.0 (176.3-271.6)	0.011
Zinc (mg)	7.0 (6.0- 8.3)	6.8 (5.9-7.9)	7.1 (6.1-8.5)	<0.001
Iron (mg)	16.6 (12.0- 23.4)	16.5 (11.8-23.1)	16.6 (12.1-23.6)	0.295
Supplement use (n (%))	302 (20.6)	80 (17.1)	222 (22.3)	0.021

[#]: Values are median (interquartile range) or number (percentage). HHcy, hyperhomocysteinemia.

Table 3 β (95% CIs) and ORs (95% CIs) of dietary intakes of antioxidant vitamins and homocysteine levels among the middle-aged and older hypertensive participants

	tHcy	Q1	Q2	Q3	Q4	$P_{\text{for trend}}$
Vitamin C (mg/d)		< 91.2	91.2-153.5	153.6-240.9	≥ 240.9	
Cases (%)		132 (36.1)	122 (33.3)	104 (28.4)	111 (30.2)	
Crude Model	-0.037 (-0.063, -0.011)	Ref.	0.89 (0.65, 1.20)	0.70 (0.52, 0.96)	0.77 (0.56, 1.05)	0.071
Model I	-0.031 (-0.055, -0.006)	Ref.	0.92 (0.66, 1.27)	0.64 (0.46, 0.90)	0.55 (0.34, 0.89)	0.048
Model II	-0.050 (-0.084, -0.016)	Ref.	0.82 (0.57, 1.16)	0.49 (0.33, 0.74)	0.40 (0.22, 0.74)	0.001
Vitamin E (mg/d)		< 19.3	19.3-25.0	25.1-31.9	≥ 31.9	
Cases (%)		122 (33.6)	126 (34.2)	113 (30.9)	108 (29.3)	
Crude Model	-0.035 (-0.084, 0.014)	Ref.	1.03 (0.76, 1.40)	0.88 (0.65, 1.20)	0.82 (0.60, 1.12)	0.139
Model I	-0.007 (-0.053, 0.039)	Ref.	1.10 (0.79, 1.53)	0.96 (0.69, 1.34)	0.95 (0.68, 1.33)	0.618
Model II	-0.009 (-0.055, 0.036)	Ref.	1.12 (0.80, 1.57)	0.95 (0.68, 1.33)	0.91 (0.65, 1.28)	0.412
Carotenes (mg/d)		< 1.78	1.78-3.30	3.31-5.61	≥ 5.61	
Cases (%)		118 (32.7)	136 (37.0)	99 (26.8)	116 (31.6)	
Crude Model	-0.024 (-0.045, -0.003)	Ref.	1.21 (0.89, 1.64)	0.76 (0.55, 1.04)	0.95 (0.70, 1.30)	0.315

continued on next page

Table 3 (*continued*)

	tHcy	Q1	Q2	Q3	Q4	$P_{\text{for trend}}$
Model I	-0.022 (-0.042, -0.003)	Ref.	1.22 (0.88, 1.69)	0.72 (0.51, 1.01)	0.93 (0.67, 1.30)	0.254
Model II	-0.022 (-0.052, 0.009)	Ref.	1.18 (0.84, 1.68)	0.68 (0.46, 1.02)	0.76 (0.43, 1.35)	0.159
Retinol (μg/d)		< 78.0	78.0-138.3	138.4-283.6	≥ 283.6	
Cases (%)		147 (40.2)	133 (36.3)	91 (24.9)	98 (26.7)	
Crude Model	-0.030 (-0.046, -0.015)	Ref.	0.85 (0.63, 1.15)	0.49 (0.36, 0.68)	0.54 (0.40, 0.74)	0.001
Model I	-0.027 (-0.041, -0.012)	Ref.	0.84 (0.61, 1.16)	0.50 (0.35, 0.70)	0.55 (0.40, 0.77)	0.003
Model II	-0.021 (-0.041, -0.002)	Ref.	0.90 (0.65, 1.25)	0.61 (0.42, 0.86)	0.86 (0.56, 1.32)	0.951
Lutein (mg/d)		< 5.22	5.22-9.48	9.49-14.82	≥ 14.82	
Cases (%)		123 (33.7)	120 (32.8)	114 (31.1)	112 (30.4)	
Crude Model	-0.028 (-0.053, -0.004)	Ref.	0.96 (0.71, 1.31)	0.89 (0.65, 1.21)	0.86 (0.63, 1.17)	0.311
Model I	-0.024 (-0.046, -0.001)	Ref.	0.98 (0.71, 1.37)	0.84 (0.61, 1.17)	0.87 (0.62, 1.22)	0.331
Model II	-0.025(-0.063, 0.013)	Ref.	0.92 (0.65, 1.31)	0.76 (0.51, 1.14)	0.66 (0.37, 1.19)	0.138

Model I: Adjusted for age, sex ('male' as the reference) BMI.

Model II: Adjusted for variables in Model I and further adjusted for sedentary time ('< 3 h/d' as the reference), the intakes of folate, vitamin B6 and vitamin B12, supplement use ('no' as the reference) and the history of cardiovascular events ('no' as the reference).

Figure Legends:

Fig. 1 Restricted cubic spline analyses illustrating the shapes of multivariable association between dietary vitamin C intake (A), or dietary retinol intake (B) and HHcy. Adjusted for age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin B_6 and vitamin B_{12}, the history of cardiovascular events ('no' as the reference).

Fig. 2 ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to vitamin C, retinol intake in subgroups, stratified by sex (A), BMI (B). Adjusted for age, sex (except sex subgroup), BMI (except BMI subgroup), sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin B6 and vitamin B12, the history of cardiovascular events ('no' as the reference).

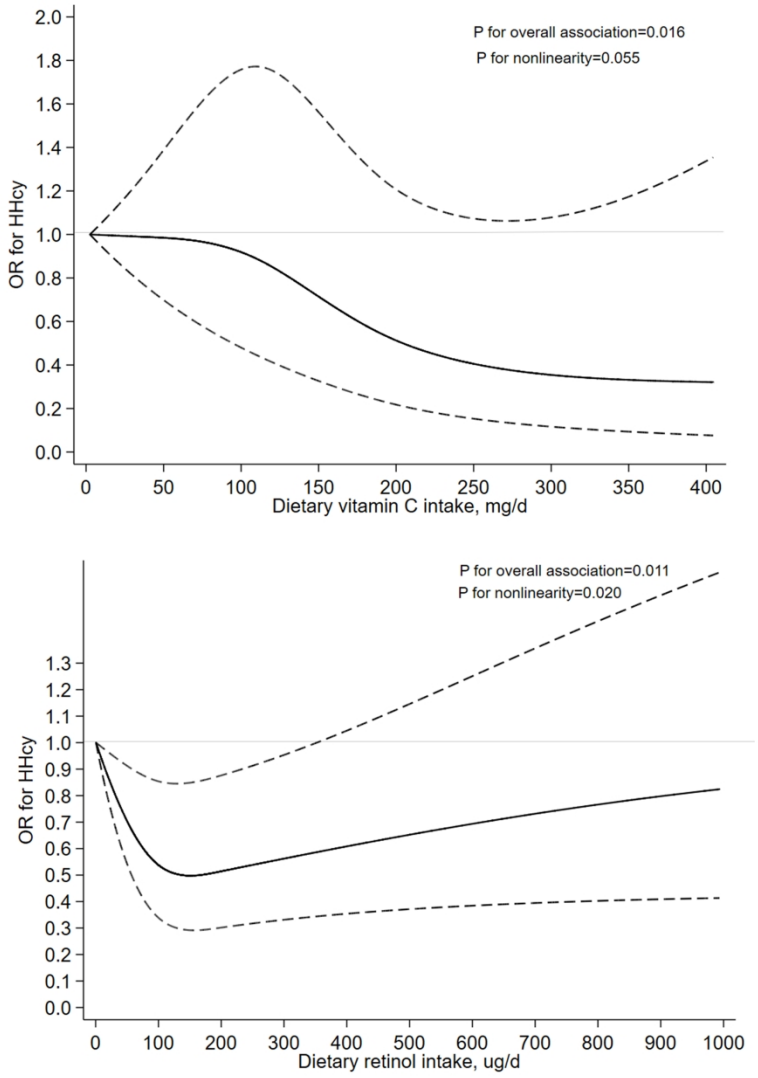


Fig 1. Restricted cubic spline analyses illustrating the shapes of multivariable association between dietary vitamin C intake (A), or dietary retinol intake (B) and HHcy. Adjusted for age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin B6 and vitamin B12, the history of cardiovascular events ('no' as the reference).

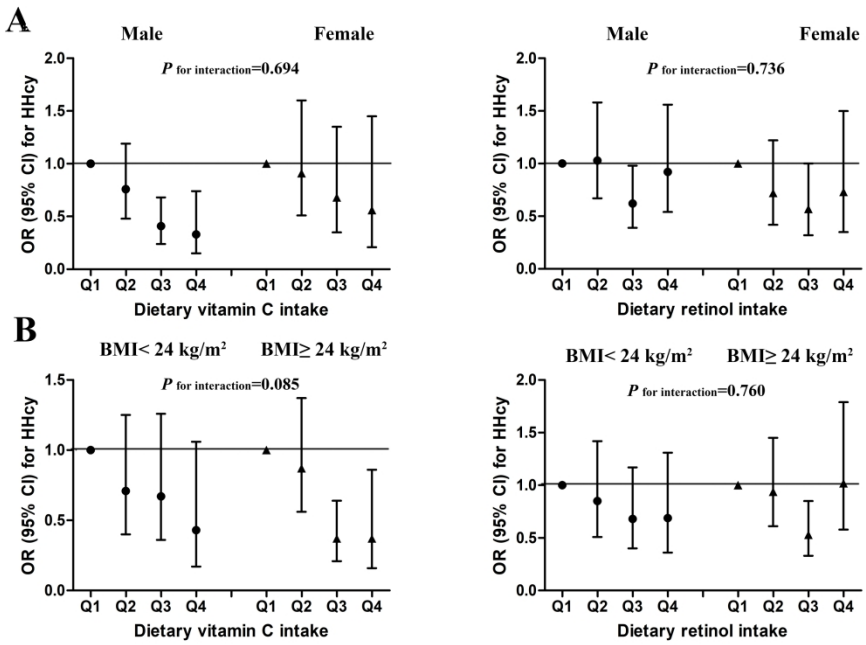


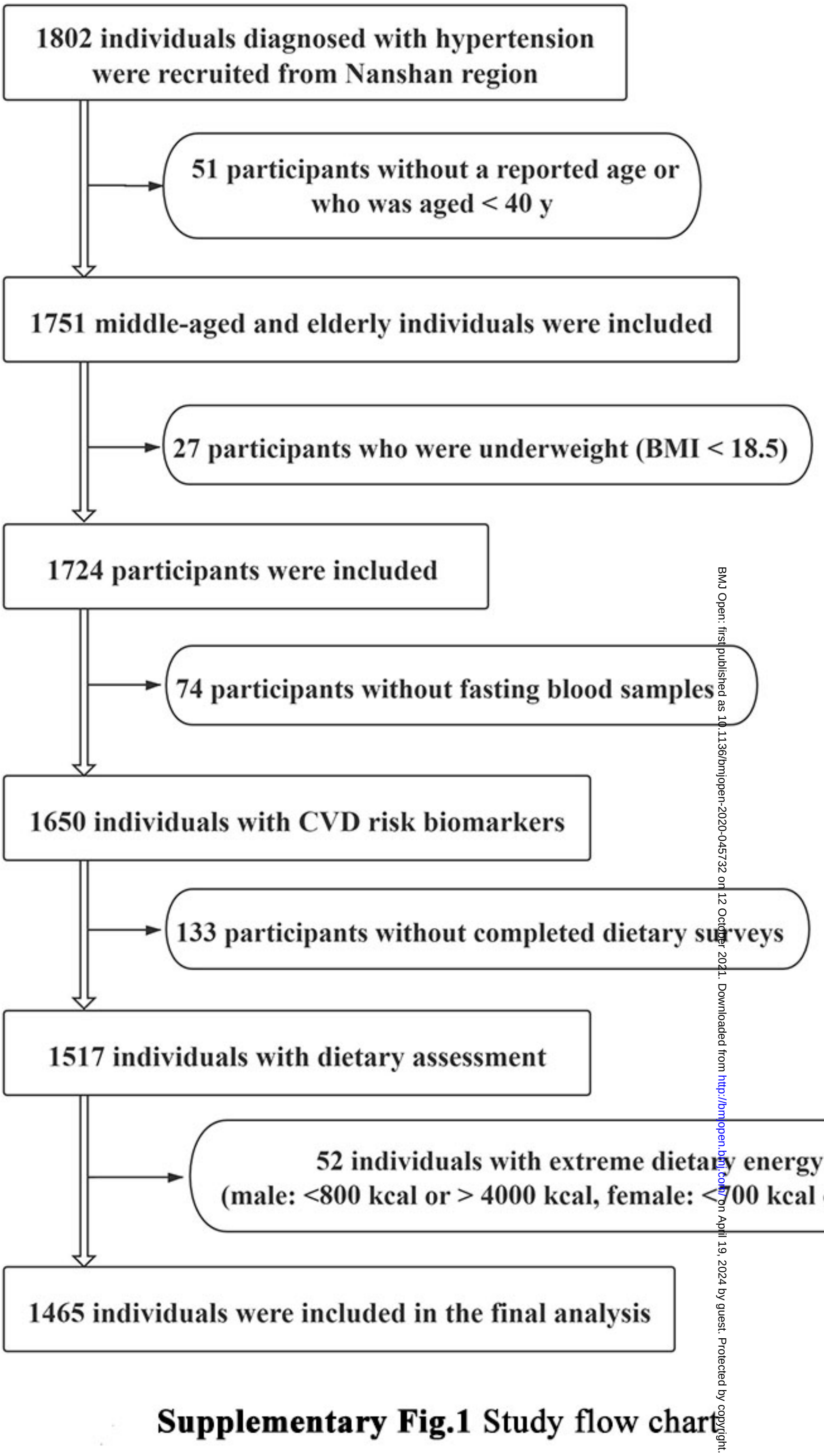
Fig 2. ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to vitamin C, retinol intake in subgroups, stratified by sex (A), BMI (B). Adjusted for age, sex (except sex subgroup), BMI (except BMI subgroup), sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin B6 and vitamin B12, the history of cardiovascular events ('no' as the reference).

289x199mm (300 x 300 DPI)

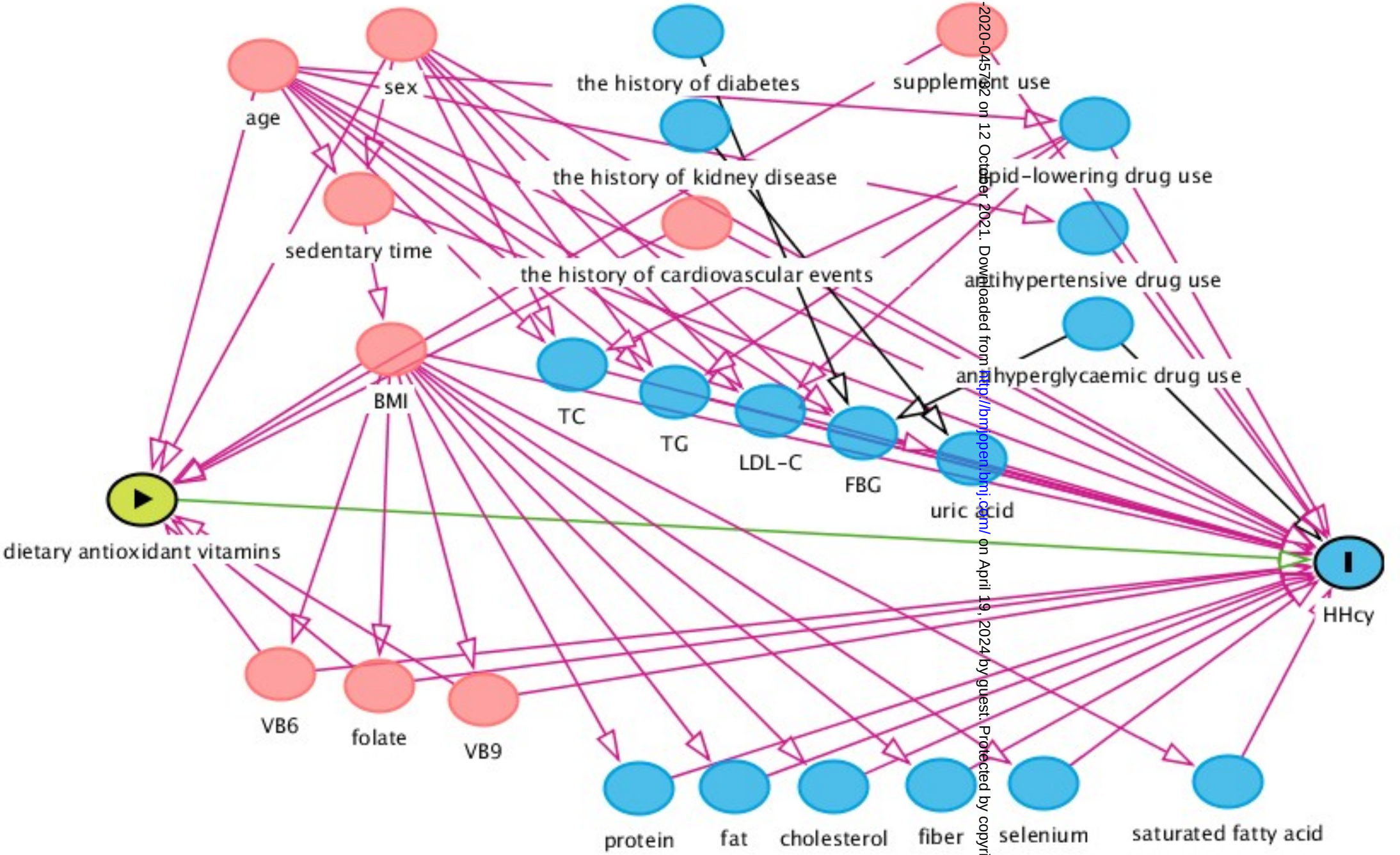
Supplementary Table 1 Sensitivity analyses of ORs (95% CIs) of antioxidant vitamins intake for hyperhomocysteinemia prevalence in multivariable-adjusted model among the patients with hypertension [#]

	Q1	Q2	Q3	Q4	$P_{\text{for trend}}$
Without cardiovascular events (n=1245)					
Vitamin C	1.00 (Ref.)	0.92 (0.63, 1.35)	0.54 (0.35, 0.84)	0.41 (0.21, 0.80)	0.003
Vitamin E	1.00 (Ref.)	1.14 (0.79, 1.64)	0.95 (0.66, 1.39)	0.90 (0.62, 1.31)	0.425
Carotenes	1.00 (Ref.)	1.25 (0.85,1.84	0.67 (0.43, 1.04)	0.77 (0.41, 1.44)	0.178
Retinol	1.00 (Ref.)	1.05 (0.73, 1.50)	0.61 (0.43, 0.94)	0.78 (0.50, 1.34)	0.836
Lutein	1.00 (Ref.)	1.04 (0.71, 1.53)	0.74 (0.48, 1.14)	0.63 (0.33, 1.20)	0.104
Without tHcy-lowering drug-using (n=1360)					
Vitamin C	1.00 (Ref.)	0.85 (0.58, 1.24)	0.49 (0.31, 0.78)	0.44 (0.22, 0.86)	0.007
Vitamin E	1.00 (Ref.)	1.07 (0.74, 1.55)	0.98 (0.68, 1.42)	0.95 (0.66, 1.37)	0.685
Carotenes	1.00 (Ref.)	1.27 (0.87, 1.85)	0.69 (0.44, 1.07)	0.67 (0.35, 1.28)	0.090
Retinol	1.00 (Ref.)	0.84 (0.59, 1.20)	0.56 (0.38, 0.84)	0.85 (0.54, 1.35)	0.837
Lutein	1.00 (Ref.)	0.88 (0.60, 1.29)	0.74 (0.47, 1.15)	0.60 (0.31, 1.16)	0.115

[#]: Adjusted for age, sex, BMI, sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin B_6 and vitamin B_{12}.



Supplementary Fig.1 Study flow chart



Supplementary Figure 2 Directed acyclic graph drawn in the DAGitty programme to identify the minimum set of confounders (depicted in red) to enter the adjusted model to examine the association between dietary intakes of antioxidant vitamins and HHcy

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	# 1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	# 2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	# 3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	# 4
Methods			
Study design	4	Present key elements of study design early in the paper	# 4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	# 4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	# 4-5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	# 5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	# 5-7
Bias	9	Describe any efforts to address potential sources of bias	# *7*
Study size	10	Explain how the study size was arrived at	# 4-5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	# 6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	# 7-8
		(*b*) Describe any methods used to examine subgroups and interactions	# 7-8
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	# 7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	# 8
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	# 5
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	# 8
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	# 8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	# 8-9
		(*b*) Report category boundaries when continuous variables were categorized	# 8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	# 9
Discussion			
Key results	18	Summarise key results with reference to study objectives	# 9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	# 12-13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	# 10-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	# 13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	# 13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.