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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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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) ($P_{\text{for trend}}=0.005$), 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 ($P_{\text{for trend}}=0.488$). 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

61 older adults with hypertension.

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

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

67 68 **Introduction**

69 Increasing evidence has shown that elevated total homocysteine (tHcy) levels are
70 associated with atherosclerotic cardiovascular diseases, stroke, peripheral arterial
71 occlusive disease, and venous thrombosis (1, 2). Hyperhomocysteinemia (HHcy)
72 (tHcy ≥ 15 $\mu\text{mol/L}$) (3), the result of a disturbed methionine metabolism, may lead to
73 an enhancement of the adverse effects of risk factors like hypertension on human
74 health (4). Hypertension is a major risk factor for cardiovascular and chronic kidney
75 disease and a major cause of the global burden of disease and mortality (5, 6). It was
76 estimated that the prevalence of hypertension in China in 2015 was 23.2%, and about
77 245 million Chinese adults had hypertension (7). Notably, the incidence of
78 hypertension with hyperhomocysteinemia, or 'H-type hypertension', is significantly
79 higher compared with other countries, representing 75% of Chinese patients with
80 hypertension (8). Thus, the nutritional treatment focus on the attempt to reduce
81 cardiovascular risk by reducing tHcy is important, particularly among patients with
82 hypertension (9).

83 Increasing age, male sex, genetic factors, consumption of alcohol or tobacco,
84 kidney function and physical activity are some of the factors associated with tHcy
85 levels (10). Epidemiological studies and clinical trials have indicated that folate,
86 vitamin B₁₂ and vitamin B₆ status, well-known predictors of tHcy, are important for
87 tHcy metabolism. The latest meta-analysis demonstrated that a lower risk of stroke
88 and overall cardiovascular disease (CVD) with folic acid supplementation, which may
89 partly contribute to the decrease of tHcy level (11). Folate, a key factor of tHcy
90 metabolism, is very sensitive to free radicals (12). There are many studies have shown

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3
4 91 the antioxidant vitamins may influence the tHcy levels for the protective effect of
5 92 folate from oxidation. Plasma levels or dietary intake of vitamin C (VC), vitamin E
6 93 (VE) or β -carotene was inversely associated with tHcy levels, however, the findings
7 94 were not consistent (13-15). Of note, the association has never been investigated
8 95 among the hypertensive population.

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

99 **Methods**

100 *Study design and population*

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

115 We collected the data of 1802 participants, and excluded 51 participants whose age
116 was not reported or who were aged ≤ 40 years and 27 underweight participants (body
117 mass index (BMI) < 18.5 kg/m²), as well as participants for whom fasting blood
118 samples (n = 74) and complete dietary surveys (n = 133) were not available. We also
119 excluded participants with an extreme dietary energy intake (male: < 800 kcal or $>$
120 4000 kcal, female: < 700 kcal or > 3500 kcal) (n = 52). Finally, 1465 participants

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3
4 121 were included in the analysis (**Supplementary Figure.1**).

5
6 122 ***Patient and public involvement***

7
8 123 The development of standardised form is in response to the public health need of
9
10 124 preventing stroke among hypertension population. Patients and the public were not
11
12 125 involved in the design of the study. The results of our study will be disseminated
13
14 126 through open access publications.

15
16 127 ***Dietary assessment***

17
18 128 The researcher-administered FFQ consisted of 92 food items, which were assembled
19
20 129 into 7 food groups: cereals, vegetables, fruits, beans, animal foods, nuts and
21
22 130 beverages. The FFQ was based on the national FFQ used in the 2010-2012 China
23
24 131 National Nutrition and Health Survey according to the Chinese Nutrition and Health
25
26 132 Surveillance in 2010-2012 (16). Participants were asked to recall the consumption of
27
28 133 each item during the past year, including the type of food, frequency and amount.
29
30 134 Food weight maps were available for participants to estimate their portion size.
31
32 135 Primary data obtained from FFQs were doubly entered into the EpiData 3.0 software
33
34 136 by two trained technicians to verify accuracy. Dietary energy and other nutrients were
35
36 137 calculated based on the Chinese Food Composition Database (17, 18). Dietary intake
37
38 138 of VC, VE, carotenes (consist of α -carotene, β -carotene and β -cryptoxanthin), and
39
40 139 retinol were calculated based on the Chinese Food Composition Table 2002 (17).
41
42 140 Dietary intake of lutein was calculated based on the food composition table of
43
44 141 vegetables, fruits, eggs and nuts that contain large amounts of lutein (Chinese Dietary
45
46 142 Reference Intakes 2013). The intake of all dietary nutrients and carotenoids was
47
48 143 adjusted for energy using the residual method (19). The second FFQ was conducted 3
49
50 144 months after the completion of the first FFQ among 108 participants. The intra-class
51
52 145 correlation coefficients of two administrations of FFQ for nutrients ranged from 0.044
53
54 146 (iodine) to 0.562 (phosphorus) and were all statistically significant, except for iodine.

54
55 147 ***Assessment of other covariates***

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57 148 Participants' socio-demographic characteristics (e.g., age and sex), lifestyle factors
58
59 149 (e.g., sedentary time), history of chronic diseases, and medication and supplement use
60
150 status were collected. Sedentary time consisted of time spent watching TV and sitting,

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4 151 which were combined into one variable with 2 categories, < 3 h/d and \geq 3 h/d, based
5
6 152 on a median sedentary time of 3 h/d. The history of cardiovascular events including
7
8 153 coronary heart disease, cerebral haemorrhage, cerebral thrombosis, and the history of
9
10 154 diabetes and kidney disease were recorded for each participant. The prescription use
11
12 155 status was classified into 2 groups (yes or no) corresponding to whether the
13
14 156 participant was taking any type or quantity of drugs, including antihypertensive drugs,
15
16 157 antihyperglycaemic drugs, lipid-lowering drugs or tHcy-lowering drugs. Supplement
17
18 158 use consisted of vitamin A, retinol, B vitamins, VC, VE and folic acid was included.

19 159 *Anthropometric measurements*

20
21 160 Height and weight and waist circumference (WC) were measured by specialists. BMI
22
23 161 was calculated as weight (kg) / height (m²). Systolic blood pressure (SBP) and
24
25 162 diastolic blood pressure (DBP) were measured from the right arm of participants in a
26
27 163 seated position after a sufficient rest period using a mercury sphygmomanometer in
28
29 164 the morning. Blood pressure was measured manually and recorded as the average of
30
31 165 three measurements.

32 166 *Laboratory tests and outcomes*

33
34 167 Fasting blood samples were collected from the participants at CHSCs and transported
35
36 168 under refrigerated conditions to a clinical laboratory of the Nanshan Centre for
37
38 169 Chronic Disease Control on the same day. Blood samples were collected through
39
40 170 deposition and centrifugation for ten minutes at 3000 s/min at room temperature. The
41
42 171 concentrations of plasma tHcy, fasting plasma glucose (FPG), total cholesterol (TC),
43
44 172 triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), uric acid and
45
46 173 creatinine were assessed on the day of blood collection using enzymatic methods via
47
48 174 an auto-analyser (HITACH 7080). The inter-day quality control assessments met the
49
50 175 standard during the analysis. HHcy was defined as plasma tHcy concentration \geq 15
51
52 176 $\mu\text{mol/L}$.

53 177 *Statistical analysis*

54
55 178 Demographic characteristics were described by means \pm SDs for normally distributed
56
57 179 data, medians (interquartile ranges, IQRs) for non-normally distributed data and
58
59 180 numbers (percentages) for categorical data. The differences between males and
60

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

184 The associations between dietary antioxidant vitamins intake and the prevalence of
185 HHcy were analyzed using multiple logistic regression models, with the lowest
186 quartile as the reference category. Potential confounders related to dietary intake of
187 antioxidant vitamins and HHcy reported in previous literature were chosen in
188 multivariable analyses. The first model was adjusted for age, sex ('male' as the
189 reference) and BMI. The second model was further adjusted for sedentary time ('< 3
190 h/d' as the reference), supplement use ('no' as the reference), dietary intake of
191 protein, fat, cholesterol, fiber, folic acid, vitamin B₆ and vitamin B₁₂,
192 saturated fatty acid, selenium, and the levels of TC, TG, LDL-C, FPG, uric acid and
193 creatinine, which were .antihypertensive drug use (yes), antihyperglycaemic drug use
194 (yes), and lipid-lowering drug use (yes). The third model was further adjusted for the
195 history of cardiovascular events and kidney disease ('no' as the reference),
196 antihypertensive drug use ('no' as the reference), antihyperglycaemic drug use ('no'
197 as the reference), and lipid-lowering drug use ('no' as the reference). Linear trends
198 were tested by creating a continuous variable for dietary antioxidant vitamins intake
199 using the median value for each quartile. The sensitive analyses between dietary
200 antioxidant vitamins intake and HHcy prevalence were applied among the population
201 who had never suffered cardiovascular events, or among the population who never
202 use the tHcy-lowering drug.

203 To further explore the nonlinearity of the relationship between dietary antioxidant
204 vitamins intake and high tHcy, we used restricted cubic spline with 4 knots at the 5th,
205 35th, 65th and 95th percentiles of dietary antioxidant vitamins intake.

206 To evaluate the modification effect by some potential prevalence factors of HHcy,
207 including sex (male or female) and BMI (<24 or ≥24 kg/m²), stratified analyses were
208 conducted by these potential factors and estimated *P* values for interaction terms.

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

211 Results

212 In this cross-sectional study, 1465 participants (male: 729, female: 736) were
213 included in the analysis, and the mean \pm SD of age and BMI was 62.0 ± 10.7 years,
214 and 24.9 ± 3.5 kg/m². The tHcy level of the participants was 14.63 ± 9.06 μ mol/L, and
215 the number (percentage) of HHcy was 469 (32.0%). The participants with HHcy were
216 older and more male, and have higher WC, SBP, tHcy, uric acid, creatinine, and
217 higher percentages of history of cardiovascular events (including
218 coronary heart disease, cerebral hemorrhage, cerebral thrombosis) and kidney disease,
219 and have lower FPG level (all $P < 0.05$) (**Table 1**).

220 The dietary intake of nutrients were shown in **Table 2**. The median (IQR) of
221 dietary antioxidant vitamins intake were as follows: VC 153.5 (91.2, 240.9) mg/d, VE
222 25.0 (19.3, 31.9) mg/d, carotenes 3.3 (1.8, 5.6) mg/d, retinol 138.3 (78.0, 283.6) μ g/d,
223 and lutein 9.5 (5.2, 14.8) mg/d. Among the participants with HHcy, the intake of
224 carbohydrate was higher, and the intake of fruit, protein, fibre, retinol, folic acid,
225 vitamin B₁₂, saturated fatty acid, selenium, magnesium, zinc was lower, and the
226 percentage of supplement use was lower (all $P < 0.05$).

227 The association between dietary antioxidant vitamins intake and the HHcy
228 prevalence are shown in **Table 3**. After adjusting for age, sex and BMI, the ORs (95%
229 CIs) for HHcy prevalence across quartiles of VC intake were 1.00, 0.92 (0.66, 1.27),
230 0.64 (0.46, 0.90), and 0.55 (0.34, 0.89) ($P_{\text{for trend}} = 0.048$); the ORs (95% CIs) across
231 quartiles of retinol intake were 1.00, 0.84 (0.61, 1.16), 0.50 (0.35, 0.70), and 0.55
232 (0.40, 0.77) ($P_{\text{for trend}} = 0.003$). In the fully adjusted model, the significant association
233 was found in the third and highest quartile of VC intake, and the ORs (95% CIs) were
234 0.53 (0.34, 0.82) and 0.43 (0.23, 0.82) ($P_{\text{for trend}} = 0.005$); the significant association
235 was only found in the third quartile of retinol intake, and the ORs (95% CIs) was 0.50
236 (0.33, 0.76) ($P_{\text{for trend}} = 0.488$). However, the non-significant association between
237 dietary intake of VE, carotenes and lutein and HHcy prevalence was found. The
238 results were similar in the sensitivity analyses that excluded participants with
239 cardiovascular events or who use the tHcy-lowering drug (see **Supplementary Table**
240 **1**).

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

249 In stratified analyses, the association between dietary VC and retinol intake and
250 HHcy prevalence were not significantly modified by sex (male or female), BMI (<24
251 or ≥ 24 kg/m²) (all P for interaction were >0.05) (**Figure.2**). Similar results of stratified
252 analyses of carotenes, lutein and VE were not shown.

253 Discussion

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

260 Numerous studies have suggested that HHcy may be a modifiable risk factor for
261 CVD, especially for stroke. In the past decades, there were many clinical trials aimed
262 to show the effect of folic acid and vitamins B₁₂ and B₆ supplement on lowering the
263 level of tHcy, however, the negative results were found in most studies (20). The
264 potential reason may be correlated with the harmful effect of unmetabolized excessive
265 folate or the cyanide and thiocyanate from excessive cyanocobalamin. In China,
266 where folate fortification has not been implemented, folic acid significantly reduced
267 the risk of stroke was observed from the China Stroke Primary Prevention Trial
268 (CSPPT) (21). Thus, appropriate B vitamins therapy is of great importance for
269 lowering tHcy level in stroke prevention.

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

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4 271 sensitive to oxidative stress (12). In the past decades, several researches reported the
5
6 272 potential association between plasma levels or intake of different antioxidant vitamins
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8 273 and tHcy level (13, 14, 22-24). In 1999, Brude IR *et al.* observed the inverse
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10 274 association between plasma tHcy concentration and dietary intake of vegetables,
11
12 275 vitamin C and β -carotene from 41 participants (22). In addition, dietary intake of
13
14 276 retinol equivalents, β -carotene and VC were inversely correlated with plasma tHcy
15
16 277 level, after adjustment for dietary B-vitamins (14). What's more, the study focus on
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18 278 the effect of antioxidant vitamins on the plasma tHcy level in a free-living elderly
19
20 279 population found that plasma VC, rather than the intake and supplementation of VC,
21
22 280 showed a negative association with tHcy in simple regression analysis, and also found
23
24 281 that the plasma levels, as well as the intake and supplementation of vitamin E, and
25
26 282 β -carotene were not associated with tHcy (13). Similarly, the cross-sectional
27
28 283 NHANES 1999–2002 study found that dietary VC and VE intake were associated
29
30 284 with a lower prevalence of elevated blood tHcy concentration, whereas no association
31
32 285 between dietary carotenes intake and tHcy was detected (23).

33 286 Consistent with our findings, these studies have a common conclusion that VC
34
35 287 intake was inversely correlated with tHcy level. Given the report of an interaction of
36
37 288 VC and folate (25), Magana AA *et al.* found the underlying molecular mechanisms
38
39 289 that VC activates the folate-mediated one-carbon cycle in C2C12 myoblasts (26).
40
41 290 Thus, VC has been explored as an attractive factor to increase circulating levels of
42
43 291 folic acid and to reduce Hcy levels.

44 292 We found a U-shaped association between dietary retinol intake and high tHcy
45
46 293 prevalence, which was similar to the conclusion that dietary intake of retinol
47
48 294 equivalents was inversely correlated with plasma tHcy level (14). Retinol, a
49
50 295 preformed vitamin A, plays an important role in vision, cellular differentiation, and
51
52 296 proliferation, as well as the immune system regulation. In addition, there is increasing
53
54 297 evidence indicates that retinol seems to inhibit thrombosis (27) and inflammation
55
56 298 effects (28), which indicates retinol is emerging as a factor of interest to CVD.
57
58 299 Brazionis L reported that plasma retinol was a novel marker for CVD mortality in
59
60 300 Australian adults, with an inverse association between plasma retinol in the middle

301 tertile and 5-year CVD mortality (29). Similarly, a U-shaped relation between plasma
302 retinol and the risk of stroke death was examined (30). However, a nested
303 case-control study showed a significant inverse association between plasma retinol
304 and the risk of first stroke among Chinese hypertensive adults from the CSPPT (31),
305 which may due to relatively low baseline retinol concentrations (median: 67.5 $\mu\text{g}/\text{dL}$).
306 Besides, the interaction of retinol and tHcy was found (31), which showed the effect
307 was stronger among the participants whose tHcy < 10 $\mu\text{mol}/\text{L}$ than whose tHcy \geq 10
308 $\mu\text{mol}/\text{L}$.

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

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

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

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

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

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

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

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

361

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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)	<i>P</i>
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).

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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)	<i>P</i>
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)	388.7 (252.5, 577.1)	0.510
Fruit (g)	79.2 (39.7, 139.5)	75.4 (33.2, 127.5)	81.4 (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)	236.8 (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)	151.0 (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

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Table 2 (continued)

	Total	HHcy (n=469)	non-HHcy (n=996)	<i>P</i>
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)	221.3 (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	<i>P</i> _{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

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Table 3 (continued)

	Q1	Q2	Q3	Q4	<i>P</i> _{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 B₆ and vitamin B₁₂, saturated fatty acid, selenium, and the levels of TC, TG, LDL-C, FPG, uric acid and creatinine.

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5 483 **Fig. 1** Restricted cubic spline analyses illustrating the shapes of multivariable
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7 484 association between dietary vitamin C intake (A), or dietary retinol intake (B) and
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9 485 HHcy. Adjusted for age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d'
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21 491 and lipid-lowering drug use ('no' as the reference).
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23 492 **Fig. 2** ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to
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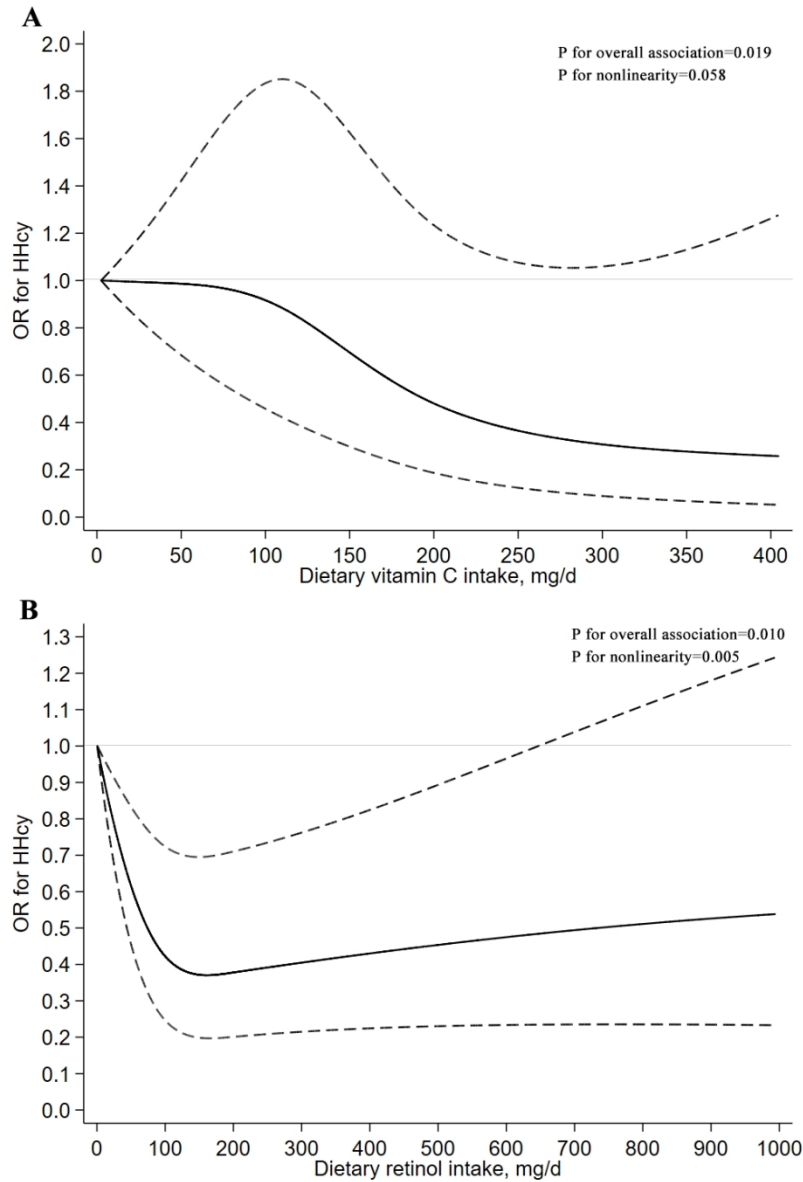


Figure 1

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).

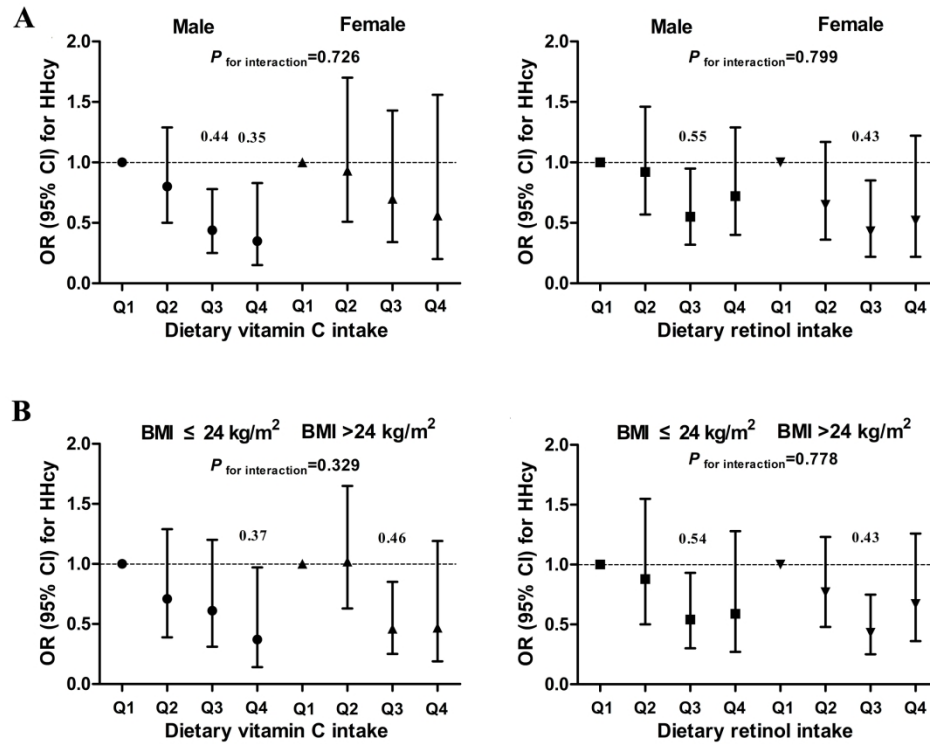
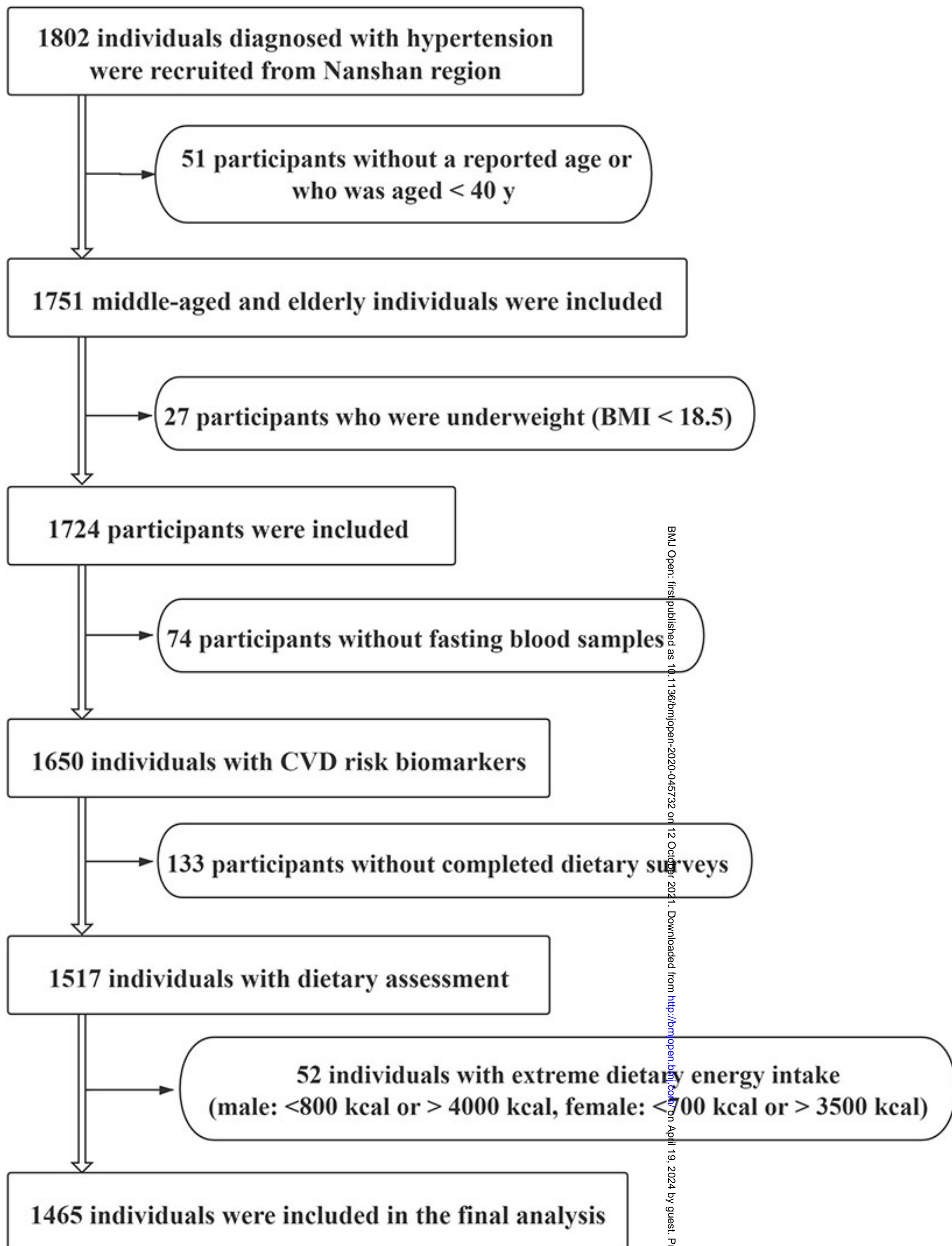


Figure 2

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).

127x96mm (600 x 600 DPI)



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	<i>P</i> 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 B₆ and vitamin B₁₂, 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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1 **Association between dietary antioxidant vitamins intake and homocysteine levels**
2 **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

61 older adults with hypertension.

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

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

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

67

68 **Introduction**

69 Increasing evidence has shown that elevated total homocysteine (tHcy) levels are
70 associated with atherosclerotic cardiovascular diseases, stroke, peripheral arterial
71 occlusive disease, and venous thrombosis (1, 2). Hyperhomocysteinemia (HHcy) (tHcy
72 $\geq 15 \mu\text{mol/L}$) (3), the result of a disturbed methionine metabolism, may lead to an
73 enhancement of the adverse effects of risk factors like hypertension on human health
74 (4). Hypertension is a major risk factor for cardiovascular and chronic kidney disease
75 and a major cause of the global burden of disease and mortality (5, 6). It was estimated
76 that the prevalence of hypertension in China in 2015 was 23.2%, and about 245 million
77 Chinese adults had hypertension (7). Notably, the incidence of hypertension with
78 hyperhomocysteinemia, or 'H-type hypertension', is significantly higher compared
79 with other countries, representing 75% of Chinese patients with hypertension (8). Thus,
80 the nutritional treatment focus on the attempt to reduce cardiovascular risk by reducing
81 tHcy is important, particularly among patients with hypertension (9).

82 Increasing age, male sex, genetic factors, consumption of alcohol or tobacco, kidney
83 function and physical activity are some of the factors associated with tHcy levels (10).
84 Epidemiological studies and clinical trials have indicated that folate, vitamin B₁₂ and
85 vitamin B₆ status, well-known predictors of tHcy, are important for tHcy metabolism.
86 The latest meta-analysis demonstrated that a lower risk of stroke and overall
87 cardiovascular disease (CVD) with folic acid supplementation, which may partly
88 contribute to the decrease of tHcy levels (11). Folate, a key factor of tHcy metabolism,
89 is very sensitive to free radicals (12-14). There are many studies have shown the
90 antioxidant vitamins may influence the tHcy levels for the protective effect of folate

1
2
3
4 91 from oxidation. Plasma levels or dietary intake of vitamin C (VC), vitamin E (VE) or
5
6 92 β -carotene was inversely associated with tHcy levels, however, the findings were not
7
8 93 consistent (15-17). Of note, the association has never been investigated among the
9
10 94 hypertensive population.

11
12 95 Therefore, this large population-based study aimed to determine the association
13
14 96 between the dietary intake of VC, VE, carotenes, retinol and lutein and the prevalence
15
16 97 of HHcy in middle-aged and older men and women with hypertension.

17 98 **Methods**

19 99 *Study design and population*

21 100 This study consecutively recruited individuals with hypertension from 60 community
22
23 101 health service centres in the Nanshan district of Shenzhen from July to September of
24
25 102 2013 using a three-stage random sampling method. In the first stage, 8 sub-districts
26
27 103 were selected in the Nanshan district; in the second stage, 6 to 8 communities were
28
29 104 selected from each sub-district using a simple random selection procedure; and in the
30
31 105 third stage, individuals with hypertension were selected from each community using
32
33 106 isometric random sampling. All subjects were of Chinese ethnicity and had lived in the
34
35 107 Nanshan district of Shenzhen for over six months. The individuals were invited to visit
36
37 108 the CHSCs, where the researcher-administered questionnaire (including the validated
38
39 109 food frequency questionnaire (FFQ)) was conducted, the anthropometric measurements
40
41 110 were recorded, and fasting blood samples were collected.

42
43 111 We collected the data of 1802 participants, and excluded 51 participants whose age
44
45 112 was not reported or who were aged ≤ 40 years and 27 underweight participants (body
46
47 113 mass index (BMI) < 18.5 kg/m²), as well as participants for whom fasting blood
48
49 114 samples (n = 74) and complete dietary surveys (n = 133) were not available. We also
50
51 115 excluded participants with an extreme dietary energy intake (male: < 800 kcal or > 4000
52
53 116 kcal, female: < 700 kcal or > 3500 kcal) (n = 52). Finally, 1465 participants were
54
55 117 included in the analysis (**Supplementary Figure 1**).

56 118 *Patient and public involvement*

58 119 The development of standardised form is in response to the public health need of
59
60 120 preventing stroke among hypertension population. Patients and the public were not

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

123 *Dietary assessment*

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

143 *Assessment of other covariates*

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

1
2
3
4 151 status was classified into 2 groups (yes or no) corresponding to whether the participant
5
6 152 was taking any type or quantity of drugs, including antihypertensive drugs,
7
8 153 antihyperglycaemic drugs, lipid-lowering drugs or tHcy-lowering drugs. Supplement
9
10 154 use consisted of vitamin A, retinol, B vitamins, VC, VE and folic acid was included.

11 155 *Anthropometric measurements*

12
13 156 Height and weight and waist circumference (WC) were measured by specialists. BMI
14
15 157 was calculated as weight (kg) / height (m²). Systolic blood pressure (SBP) and diastolic
16
17 158 blood pressure (DBP) were measured from the right arm of participants in a seated
18
19 159 position after a sufficient rest period using a mercury sphygmomanometer in the
20
21 160 morning. Blood pressure was measured manually and recorded as the average of three
22
23 161 measurements.

24 162 *Laboratory tests and outcomes*

25
26
27 163 Fasting blood samples were collected from the participants at the community health
28
29 164 service centres and transported under refrigerated conditions to a clinical laboratory of
30
31 165 the Nanshan Centre for Chronic Disease Control within 2 hours. The blood specimens
32
33 166 were collected in a 5-ml EDTA vacuum tube. Blood samples were collected through
34
35 167 deposition and centrifugation for ten minutes at 3000 r/min at room temperature. The
36
37 168 concentrations of plasma tHcy, fasting plasma glucose (FPG), total cholesterol (TC),
38
39 169 triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), uric acid and creatinine
40
41 170 were assessed on the day of blood collection using enzymatic methods via an auto-
42
43 171 analyser (HITACHI 7080). The inter-day quality control assessments met the standard
44
45 172 during the analysis. HHcy was defined as plasma tHcy concentration $\geq 15 \mu\text{mol/L}$.

46 173 *Statistical analysis*

47
48 174 Demographic characteristics were described by means \pm SDs for normally distributed
49
50 175 data, medians (interquartile ranges, IQRs) for non-normally distributed data and
51
52 176 numbers (percentages) for categorical data. The differences between males and females
53
54 177 were compared using the *t* test for normally distributed variables, the Kruskal-Wallis
55
56 178 rank test for non-normally distributed variables, and the chi-square test for categorical
57
58 179 variables.

59
60 180 Both dietary intakes of antioxidant vitamins and tHcy concentration were In-

181 transformed to improve normality before analyses and categorised into quartiles. The
182 associations between dietary antioxidant vitamins intake and the prevalence of HHcy
183 were analyzed using multiple logistic regression models, with the lowest quartile as the
184 reference category. Confounding variables were selected based on the minimal
185 sufficient adjustment recommended by the Directed Acyclic Graph, created in the
186 online software Dagitty 3.0 (**Supplementary Figure 2**). The selected potential
187 confounders included age, sex ('male' as the reference), BMI, sedentary time (< 3 h/d'
188 as the reference), the intakes of folate, vitamin B₆ and vitamin B₁₂, supplement use ('no'
189 as the reference) and the history of cardiovascular events ('no' as the reference). Linear
190 trends were tested by creating a continuous variable for dietary antioxidant vitamins
191 intake using the median value for each quartile. The sensitive analyses between dietary
192 antioxidant vitamins intake and HHcy prevalence were applied among the population
193 who had never suffered cardiovascular events, or among the population who never use
194 the tHcy-lowering drug.

195 To further explore the nonlinearity of the relationship between dietary antioxidant
196 vitamins intake and high tHcy, we used restricted cubic spline with 4 knots at the 5th,
197 35th, 65th and 95th percentiles of dietary antioxidant vitamins intake.

198 To evaluate the modification effect by some potential prevalence factors of HHcy,
199 including sex (male or female) and BMI (<24 or ≥24 kg/m²), stratified analyses were
200 conducted by these potential factors and estimated *P* values for interaction terms.

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

203 **Results**

204 In this cross-sectional study, 1465 participants (male: 729, female: 736) were
205 included in the analysis, and the mean ± SD of age and BMI was 62.0 ± 10.7 years, and
206 24.9 ± 3.5 kg/m². The tHcy levels of the participants was 14.63 ± 9.06 μmol/L, and the
207 number (percentage) of HHcy was 469 (32.0%). The participants with HHcy were older
208 and more male, and have higher WC, SBP, tHcy, uric acid, creatinine, and higher
209 percentages of history of cardiovascular events (including coronary heart disease,
210 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) $\mu\text{g/d}$, 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 B₁₂, 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

241 logistic regression analyses. The threshold analysis showed that the ORs (95% CIs) of
242 HHcy was 0.995 (0.991, 0.995) ($P=0.005$) when retinol intake was lower than 147.2
243 $\mu\text{g}/\text{d}$ and 1.000 (1.000, 1.001) ($P=0.094$) when retinol intake was more than 147.2 $\mu\text{g}/\text{d}$,
244 and the P value of log-likelihood ratio was 0.003. The non-association between
245 carotenes, lutein and VE and HHcy was not shown.

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

250 Discussion

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

257 Numerous studies have suggested that HHcy may be a modifiable risk factor for
258 CVD, especially for stroke. In the past decades, there were many clinical trials aimed
259 to show the effect of folate and vitamins B₁₂ and B₆ supplement on lowering the levels
260 of tHcy, however, the negative results were found in most studies (23). The potential
261 reason may be correlated with the harmful effect of unmetabolized excessive folate or
262 the cyanide and thiocyanate from excessive cyanocobalamin. In China, where folate
263 fortification has not been implemented, folic acid significantly reduced the risk of
264 stroke was observed from the China Stroke Primary Prevention Trial (CSPPT) (24).
265 Thus, appropriate B vitamins therapy is of great importance for lowering tHcy levels in
266 stroke prevention.

267 Notably, folate, which exists in blood and tissues mainly in a labile form, is very
268 sensitive to oxidative stress (12-14). In addition, methionine synthase and cystathionine
269 β -synthase (the key enzymes involved in methionine and homocysteine metabolism)
270 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 B₁₂ (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 μ g/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

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

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

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

349 Our study has several limitations. First, based on a cross-sectional study design, we
350 cannot draw a conclusion about causality. Second, we adjusted the dietary intake of
351 folate, B₁₂, B₆, rather than plasma concentrations, which could not eliminate
352 confounding effect. Just like Konstantinova SV (16) reported, the inverse correlation of
353 dietary retinol intake and plasma tHcy disappeared after adjustment for plasma folate
354 and vitamin B₁₂. Then, we investigated the participants whether they took dietary
355 supplements, while the detailed doses were not recorded. Therefore, we are not able to
356 eliminate the possible association between supplement use and HHcy prevalence.
357 Nevertheless, the study reported that there was some potential benefit from the
358 antioxidant supplementation on plasma tHcy concentration (28), which could stabilize
359 the positive results in this study. In addition, although some confounding factors were
360 included in the analysis, other potential confounders may exist. For instance, the

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

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

369

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379 **Contributors**

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

385 **Competing interests**

386 No, there are no competing interests for any author.

387 **Ethics approval**

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

391 **Data availability statement**

392 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)	<i>P</i>
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)	<i>P</i>
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)	388.7 (252.5, 577.1)	0.510
Fruit (g)	79.2 (39.7, 139.5)	75.4 (33.2, 127.5)	81.4 (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)	236.8 (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)	151.0 (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)	176.7 (112.8-266.9)	0.009

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Table 2 (continued)

	Total	HHcy (n=469)	non-HHcy (n=996)	<i>P</i>
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)	221.3 (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 level among the middle-aged and older hypertensive participants

	tHcy	Q1	Q2	Q3	Q4	<i>P</i> for trend
Vitamin C (mg/d)		< 91.2	91.2-153.5	153.6-240.9	\geq 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	\geq 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	\geq 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

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Table 3 (continued)

	tHcy	Q1	Q2	Q3	Q4	<i>P</i> 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).

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4 510 Figure Legends:

5 511 **Fig. 1** Restricted cubic spline analyses illustrating the shapes of multivariable
6 association between dietary vitamin C intake (A), or dietary retinol intake (B) and
7 HHcy. Adjusted for age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as
8 the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin
9 B₆ and vitamin B₁₂, the history of cardiovascular events ('no' as the reference).
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15 516 **Fig. 2** ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to
16 vitamin C, retinol intake in subgroups, stratified by sex (A), BMI (B). Adjusted for age,
17 sex (except sex subgroup), BMI (except BMI subgroup), sedentary time ('< 3 h/d' as
18 the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin
19 B₆ and vitamin B₁₂, the history of cardiovascular events ('no' as the reference).
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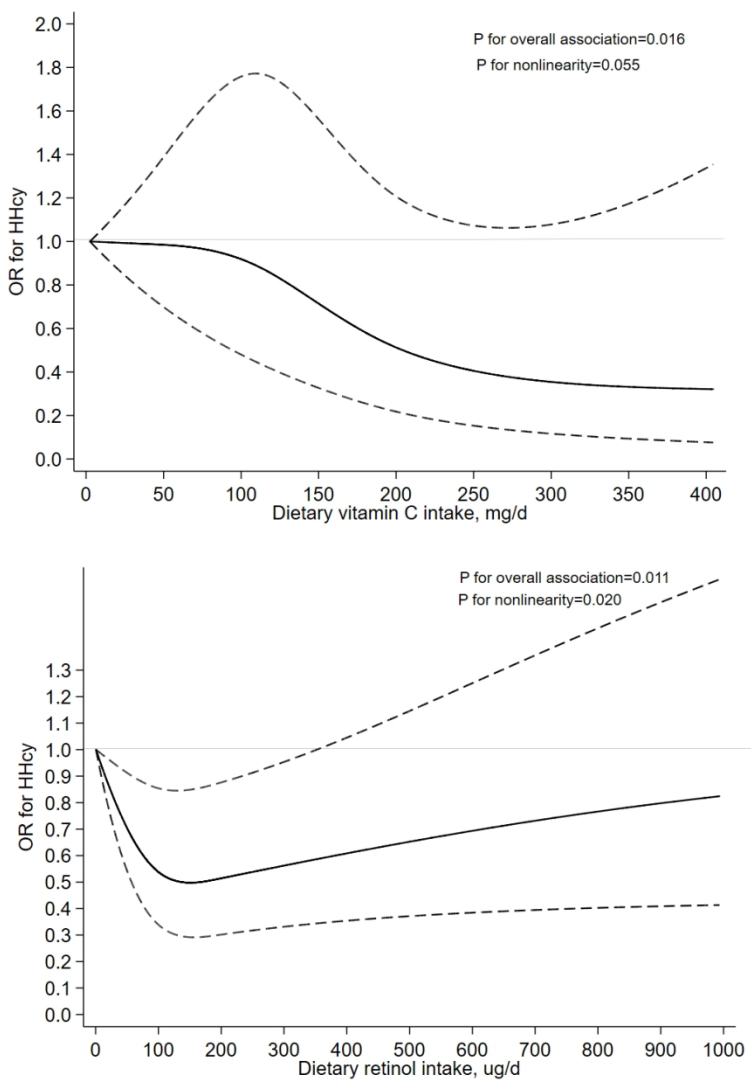


Figure 1 Restricted cubic spline analyses illustrating the shapes of multivariable association between dietary vitamin C intake (A), or dietary retinol intake (B) and HHcy. Adjusted for age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference),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

187x287mm (144 x 144 DPI)

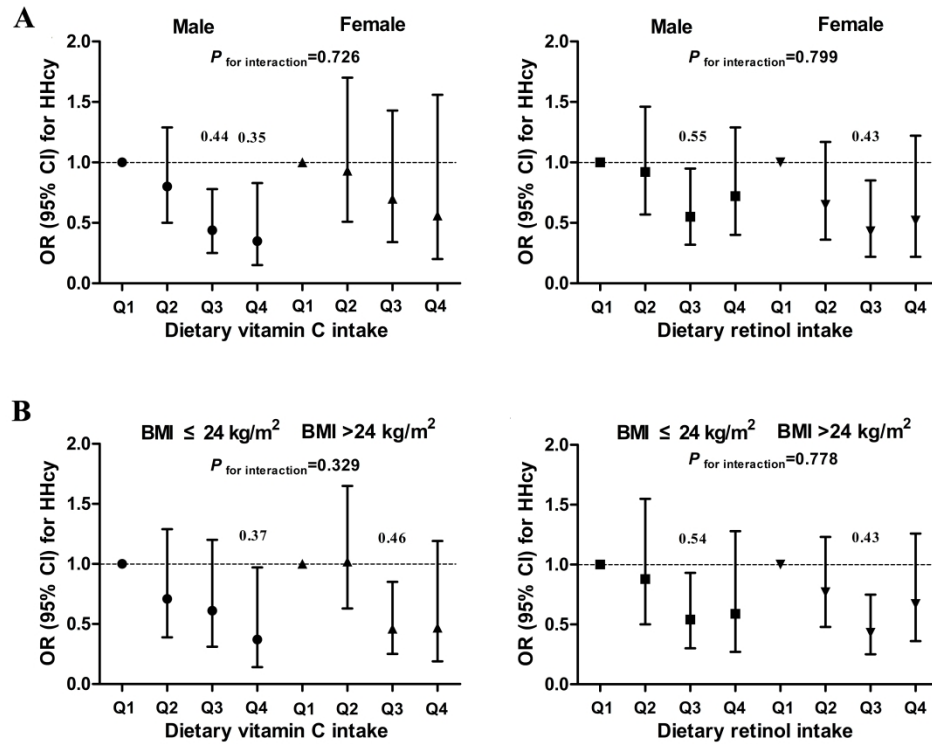
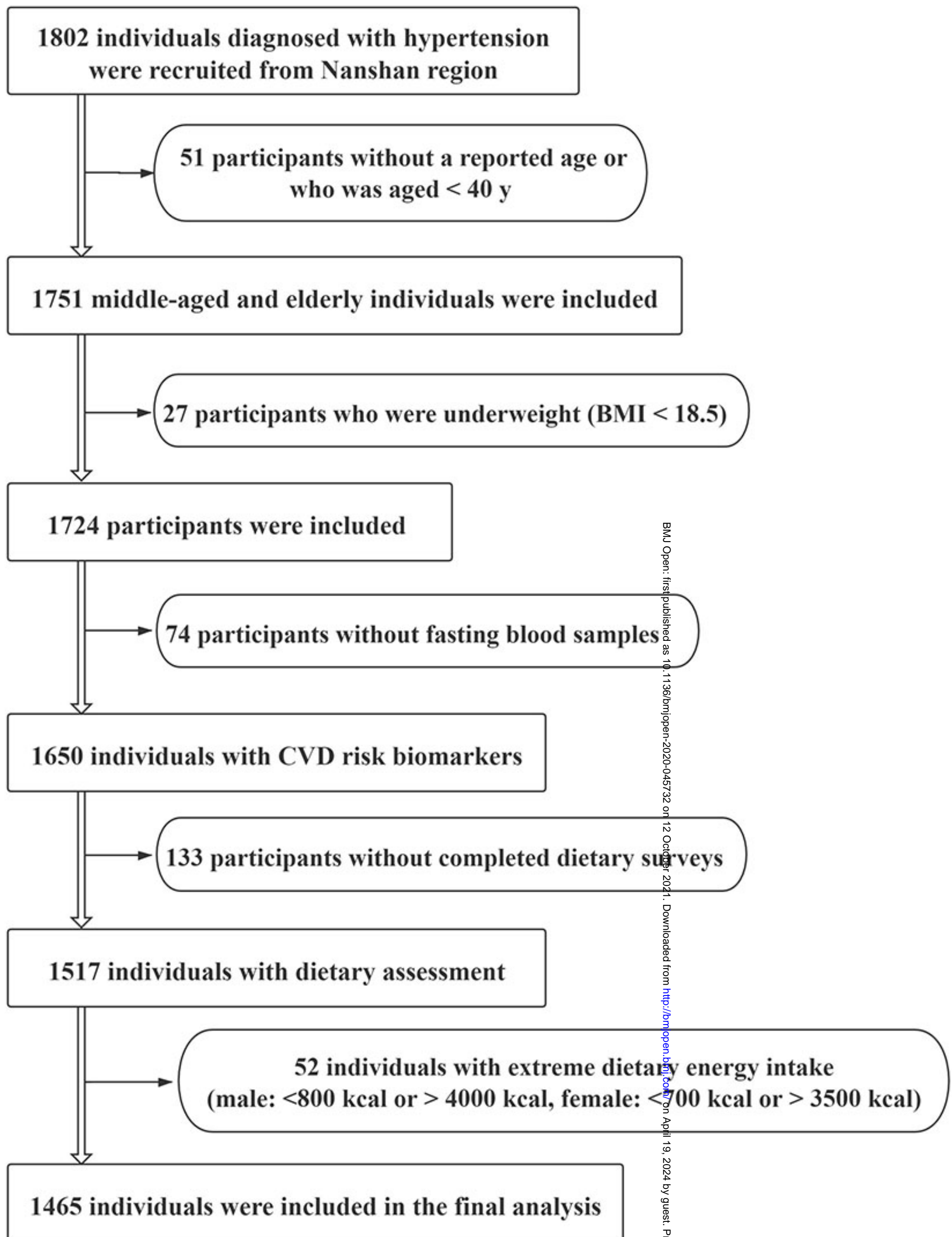
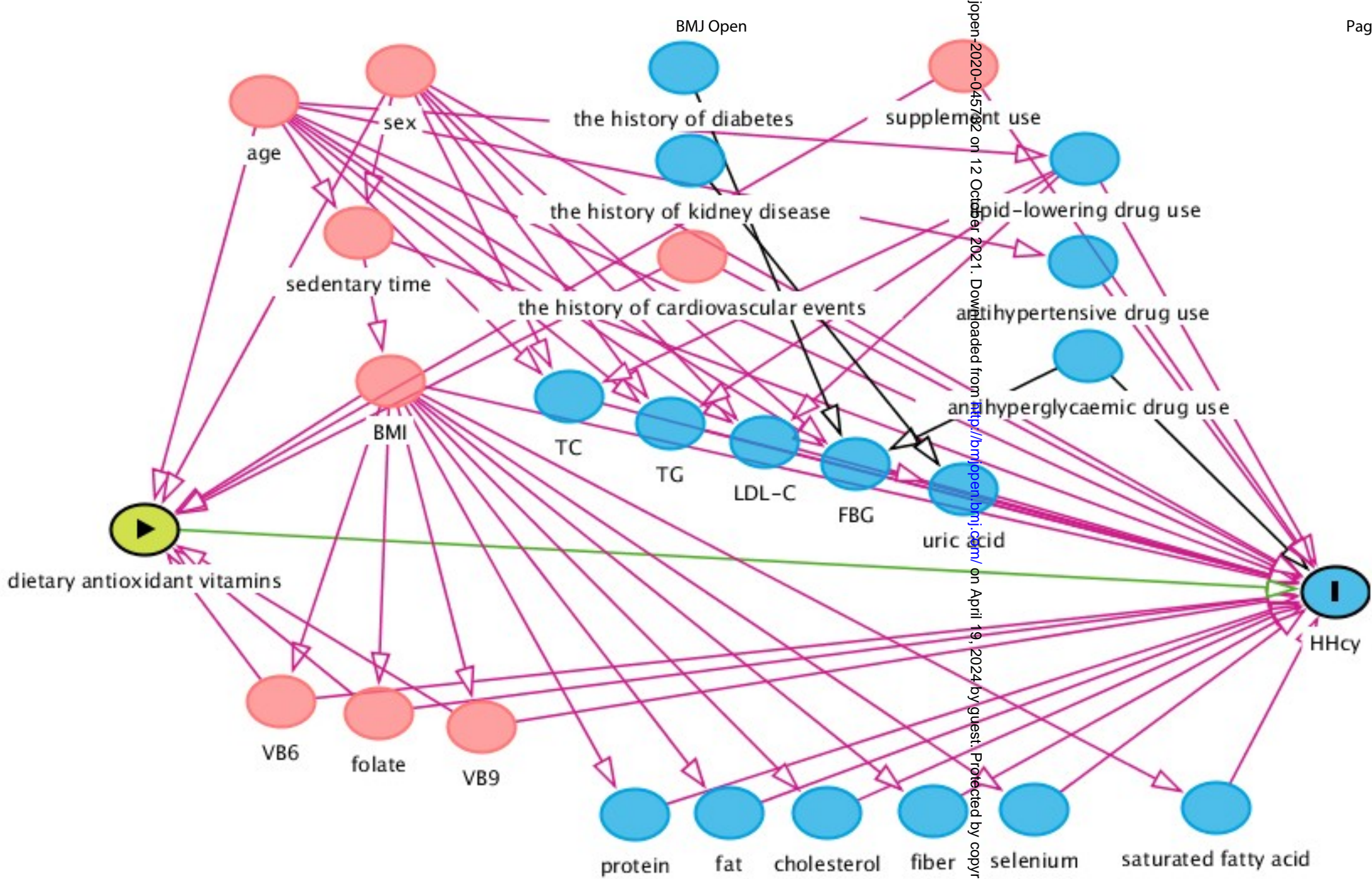


Figure 2 ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to vitamin C, retinol intake in subgroups, stratified by sex (A), BMI (B). Adjusted for age, sex (except sex subgroup), BMI (except BMI subgroup), sedentary time (< 3 h/d' as the reference), supplement use ('no' as the reference),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



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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

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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	<i>P</i> 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 B₆ and vitamin B₁₂.

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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1 **Association between dietary antioxidant vitamins intake and homocysteine levels**
2 **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 $\mu\text{mol/L}$. 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.

61 Strengths and limitations of this study:

62 1. This study focused on the risk of hyperhomocysteinemia among middle-aged and
63 older adults with hypertension.

64 2. A linear inverse association between dietary VC intake and HHcy prevalence and
65 the threshold effect of retinol on HHcy was reported in this study.

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

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

71 Introduction

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

85 Increasing age, male sex, genetic factors, consumption of alcohol or tobacco, kidney
86 function and physical activity are some of the factors associated with tHcy levels (10).
87 Epidemiological studies and clinical trials have indicated that folate, vitamin B₁₂ and
88 vitamin B₆ status, well-known predictors of tHcy, are important for tHcy metabolism.
89 The latest meta-analysis demonstrated that a lower risk of stroke and overall
90 cardiovascular disease (CVD) with folic acid supplementation, which may partly

91 contribute to the decrease of tHcy levels (11).

92 Folic acid and vitamin B₁₂, which are involved in remethylation of homocysteine to
93 methionine by methionine synthase (MS), and vitamin B₆, which acts as a cofactor in
94 the transsulfuration of homocysteine to cysteine by cystathionine β-synthase (CBS)
95 (12). Folate, a key factor of tHcy metabolism, is very sensitive to free radicals (13-15).
96 In addition, MS and CBS were strongly influenced by oxidative stress, which may be
97 associated with dietary antioxidant vitamins and Hcy levels (16,17). We hypothesized
98 that antioxidant nutrients may regulate tHcy metabolism by influencing methionine-
99 homocysteine cycle. Plasma levels or dietary intake of vitamin C (VC), vitamin E (VE)
100 or β-carotene was inversely associated with tHcy levels, however, the findings were not
101 consistent (18-20). Of note, the association has never been investigated among the
102 hypertensive population.

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

106 **Methods**

107 *Study design and population*

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

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

121 mass index (BMI) < 18.5 kg/m²), as well as participants for whom fasting blood
122 samples (n = 74) and complete dietary surveys (n = 133) were not available. We also
123 excluded participants with an extreme dietary energy intake (male: < 800 kcal or > 4000
124 kcal, female: < 700 kcal or > 3500 kcal) (n = 52). Finally, 1465 participants were
125 included in the analysis (**Supplementary Figure 1**).

126 *Patient and public involvement*

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

131 *Dietary assessment*

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

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2
3
4 151 The intra-class correlation coefficients of two administrations of FFQ for nutrients of
5 152 VC, VE, carotenes, retinol, lutein were 0.395, 0.477, 0.355, 0.551 and 0.350, and were
6
7 153 all statistically significant.

9 154 ***Assessment of other covariates***

11 155 Participants' socio-demographic characteristics (e.g., age and sex), lifestyle factors
12 156 (e.g., sedentary time), history of chronic diseases, and medication and supplement use
13 157 status were collected. Sedentary time consisted of time spent watching TV and sitting,
14 158 which were combined into one variable with 2 categories, < 3 h/d and \geq 3 h/d, based on
15 159 a median sedentary time of 3 h/d. The history of cardiovascular events including
16 160 coronary heart disease, cerebral haemorrhage, cerebral thrombosis, and the history of
17 161 diabetes and kidney disease were recorded for each participant. The prescription use
18 162 status was classified into 2 groups (yes or no) corresponding to whether the participant
19 163 was taking any type or quantity of drugs, including antihypertensive drugs,
20 164 antihyperglycaemic drugs, lipid-lowering drugs or tHcy-lowering drugs. Supplement
21 165 use consisted of vitamin A, retinol, B vitamins, VC, VE and folic acid was included.

23 166 ***Anthropometric measurements***

25 167 Height and weight and waist circumference (WC) were measured by specialists. BMI
26 168 was calculated as weight (kg) / height (m²). Systolic blood pressure (SBP) and diastolic
27 169 blood pressure (DBP) were measured from the right arm of participants in a seated
28 170 position after a sufficient rest period using a mercury sphygmomanometer in the
29 171 morning. Blood pressure was measured manually and recorded as the average of three
30 172 measurements.

32 173 ***Laboratory tests and outcomes***

34 174 Morning fasting elbow vein blood samples were required to fast overnight (at least 10
35 175 hours) were collected from the participants at the CHSCs and transported under
36 176 refrigerated conditions to a clinical laboratory of the Nanshan Centre for Chronic
37 177 Disease Control within 2 hours. The blood specimens were collected in a 5-ml EDTA
38 178 vacuum tube. Blood samples were collected through deposition and centrifugation for
39 179 ten minutes at 3000 r/min at room temperature. The concentrations of plasma tHcy,
40 180 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 $\geq 15 \mu\text{mol/L}$.

Statistical analysis

Demographic characteristics were described by means \pm 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 B₆ and vitamin B₁₂, 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 5th, 35th, 65th and 95th percentiles of dietary antioxidant vitamins intake.

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

211 including sex (male or female) and BMI (<24 or ≥ 24 kg/m²), stratified analyses were
212 conducted by these potential factors and estimated *P* values for interaction terms.

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

215 Results

216 In this cross-sectional study, 1465 participants (male: 729, female: 736) were
217 included in the analysis, and the mean \pm SD of age and BMI was 62.0 ± 10.7 years, and
218 24.9 ± 3.5 kg/m². The tHcy levels of the participants was 14.63 ± 9.06 μ mol/L, and the
219 number (percentage) of HHcy was 469 (32.0%). The participants with HHcy were older
220 and more male, and have higher WC, SBP, tHcy, uric acid, creatinine, and higher
221 percentages of history of cardiovascular events (including coronary heart disease,
222 cerebral hemorrhage, cerebral thrombosis) and kidney disease, and have lower FPG
223 level (all $P < 0.05$) (**Table 1**).

224 The dietary intakes of nutrients were shown in **Table 2**. The median (IQR) of dietary
225 antioxidant vitamins intake were as follows: VC 153.5 (91.2, 240.9) mg/d, VE 25.0
226 (19.3, 31.9) mg/d, carotenes 3.3 (1.8, 5.6) mg/d, retinol 138.3 (78.0, 283.6) μ g/d, and
227 lutein 9.5 (5.2, 14.8) mg/d. Among the participants with HHcy, the intake of
228 carbohydrate was higher, and the intake of fruit, protein, fibre, retinol, folate, vitamin
229 B₁₂, saturated fatty acid, selenium, magnesium, zinc was lower, and the percentage of
230 supplement use was lower (all $P < 0.05$).

231 The association between dietary antioxidant vitamins intake and tHcy levels are
232 shown in **Table 3**. The inverse association between VC intake and tHcy concentration
233 after ln-transformed in the fully adjusted model, and the β (95% CIs) was -0.050 (-
234 0.084, -0.016), which was consistent with the logistic regression as categorical
235 variables. In Model II, the significant association was found in the third and highest
236 quartile of VC intake, and the ORs (95% CIs) were 0.49 (0.33, 0.74) and 0.40 (0.22,
237 0.74) ($P_{\text{for trend}} = 0.001$). The retinol intake was also inversely associated with tHcy, as
238 the β (95% CIs) was -0.021 (-0.041, -0.002). After adjusting for age, sex and BMI, the
239 ORs (95% CIs) for HHcy prevalence across quartiles of retinol intake were 1.00, 0.84
240 (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

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

247 After fully adjusting for the potential confounders, the association between dietary
248 antioxidant vitamins intake and the prevalence of HHcy were shown in **Figure.1**. From
249 the cubic splines, we noted that the linear inverse trend of VC intake and HHcy
250 prevalence (P for overall association=0.016, P for nonlinearity=0.055) and the L-
251 shaped relationship of retinol intake and HHcy prevalence (P for overall
252 association=0.011, P for nonlinearity=0.020), which were consistent with the results of
253 logistic regression analyses. The threshold analysis showed that the ORs (95% CIs) of
254 HHcy was 0.995 (0.991, 0.995) ($P=0.005$) when retinol intake was lower than 147.2
255 $\mu\text{g/d}$ and 1.000 (1.000, 1.001) ($P=0.094$) when retinol intake was more than 147.2 $\mu\text{g/d}$,
256 and the P value of log-likelihood ratio was 0.003. The non-association between
257 carotenes, lutein and VE and HHcy was not shown.

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

262 Discussion

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

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

271 to show the effect of folate and vitamins B₁₂ and B₆ supplement on lowering the levels
272 of tHcy, however, the negative results were found in most studies (26). The potential
273 reason may be correlated with the harmful effect of unmetabolized excessive folate or
274 the cyanide and thiocyanate from excessive cyanocobalamin. In China, where folate
275 fortification has not been implemented, folic acid significantly reduced the risk of
276 stroke was observed from the China Stroke Primary Prevention Trial (CSPPT) (27).
277 Thus, appropriate B vitamins therapy is of great importance for lowering tHcy levels in
278 stroke prevention.

279 In the past decades, several researches reported the potential association between
280 plasma levels or intake of different antioxidant vitamins and tHcy levels (18, 19, 28-
281 30). In 1999, Brude IR *et al.* observed the inverse association between plasma tHcy
282 concentration and dietary intake of vegetables, vitamin C and β -carotene from 41
283 participants (28). In addition, dietary intake of retinol equivalents, β -carotene and VC
284 were inversely correlated with plasma tHcy levels, after adjustment for dietary B-
285 vitamins, but not after additional adjustment for plasma folate and vitamin B₁₂ (19).
286 What's more, the study focused on the effect of antioxidant vitamins on the plasma
287 tHcy levels in a free-living elderly population found that plasma VC, rather than the
288 intake and supplementation of VC, showed a negative association with tHcy in simple
289 regression analysis, and also found that the plasma levels, as well as the intake and
290 supplementation of vitamin E, and β -carotene were not associated with tHcy (18).
291 Similarly, the cross-sectional NHANES 1999–2002 study found that dietary VC and
292 VE intake were associated with a lower prevalence of elevated blood tHcy
293 concentration, whereas no association between dietary carotenes intake and tHcy was
294 detected (29). In addition, Rajesh Ullegaddi *et al.* found that significant reductions in
295 plasma homocysteine in the group with antioxidant treatment (vitamins C and E)
296 combination with B-vitamin, compared with the group with B-vitamin alone (30),
297 which support the importance of antioxidant vitamins on tHcy metabolism.

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

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

304 We found an L-shaped association between dietary retinol intake and high tHcy
305 prevalence, which meant that if the retinol intake was low, the risk of HHcy was
306 decreased as retinol intake increased, but the risk was not changed when retinol intake
307 reached certain level, which was more than 147.2 $\mu\text{g}/\text{d}$ among the participants in this
308 study. The dietary intake of retinol equivalents was inversely correlated with plasma
309 tHcy levels after adjustment for dietary B-vitamins (19). Retinol, a preformed vitamin
310 A, plays an important role in vision, cellular differentiation, and proliferation, as well
311 as the immune system regulation. In addition, there is increasing evidence indicates that
312 retinol seems to inhibit thrombosis (33) and inflammation effects (34), which indicates
313 retinol is emerging as a factor of interest to CVD. Brazionis L *et al.* reported that plasma
314 retinol was a novel marker for CVD mortality in Australian adults, with an inverse
315 association between plasma retinol in the middle tertile and 5-year CVD mortality (35).
316 Similarly, a strong association between low retinol and the risk of sudden cardiac death
317 was examined (36). In addition, a nested case-control study showed a significant
318 inverse association between plasma retinol and the risk of first stroke among Chinese
319 hypertensive adults from the CSPPT (37), which may due to relatively low baseline
320 retinol concentrations (median: 67.5 $\mu\text{g}/\text{dL}$). Besides, the interaction of retinol and tHcy
321 on CVD risk was also reported. Yu Y *et al.* (37) showed the inverse effect between
322 plasma retinol and first stroke was stronger among the participants whose tHcy < 10
323 $\mu\text{mol}/\text{L}$ than whose tHcy $\geq 10 \mu\text{mol}/\text{L}$. Olsen T *et al.* (38) found that the plasma tHcy
324 was associated with acute myocardial infarction only in the upper Vit-A tertile, and the
325 potential mechanisms may include inflammation and lipid metabolism, which may be
326 partly interpreted with the high intake of retinol (1576 μg RAE/d).

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

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

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

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

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

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

360 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, B₁₂, B₆, 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 B₁₂. 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)	<i>P</i>
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)	<i>P</i>
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)	388.7 (252.5, 577.1)	0.510
Fruit (g)	79.2 (39.7, 139.5)	75.4 (33.2, 127.5)	81.4 (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)	236.8 (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)	151.0 (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)	<i>P</i>
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)	221.3 (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 level among the middle-aged and older hypertensive participants

	tHcy	Q1	Q2	Q3	Q4	<i>P</i> for trend
Vitamin C (mg/d)		< 91.2	91.2-153.5	153.6-240.9	\geq 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	\geq 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	\geq 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	<i>P</i> 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).

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4 540 Figure Legends:

5 541 **Fig. 1** Restricted cubic spline analyses illustrating the shapes of multivariable
6 association between dietary vitamin C intake (A), or dietary retinol intake (B) and
7 HHcy. Adjusted for age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as
8 the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin
9 B₆ and vitamin B₁₂, the history of cardiovascular events ('no' as the reference).
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14 546 **Fig. 2** ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to
15 vitamin C, retinol intake in subgroups, stratified by sex (A), BMI (B). Adjusted for age,
16 sex (except sex subgroup), BMI (except BMI subgroup), sedentary time ('< 3 h/d' as
17 the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin
18 B₆ and vitamin B₁₂, the history of cardiovascular events ('no' as the reference).
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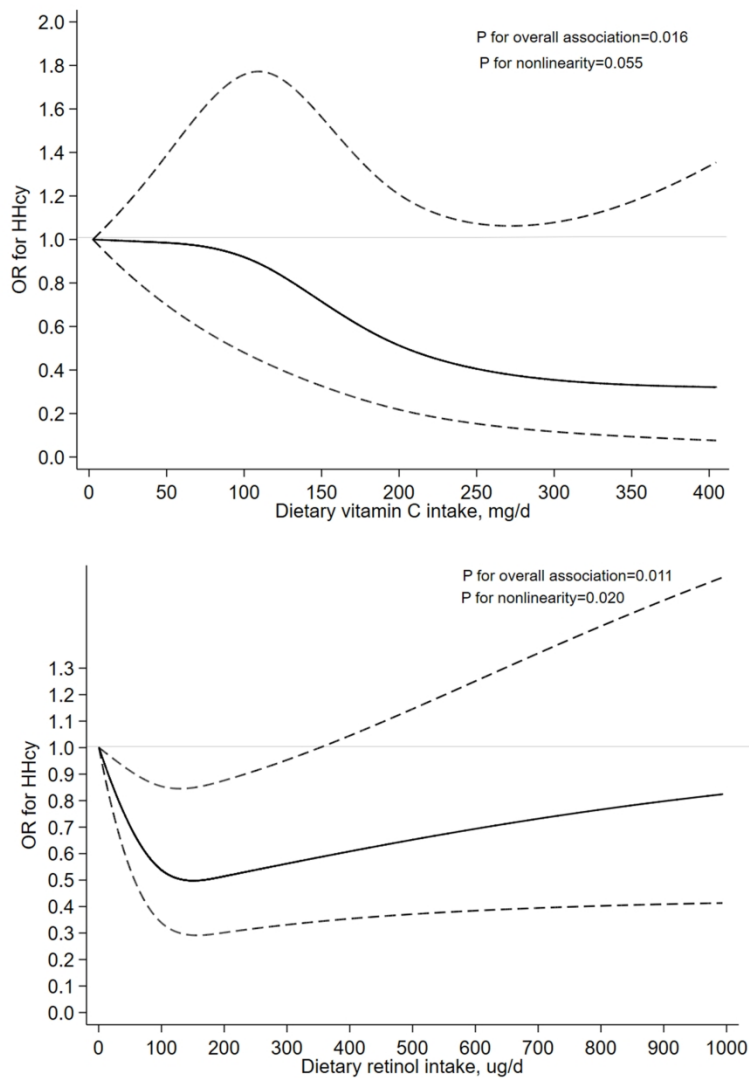


Fig. 1 Restricted cubic spline analyses illustrating the shapes of multivariable association between dietary vitamin C intake (A), or dietary retinol intake (B) and HHcy. Adjusted for age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference),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

187x287mm (300 x 300 DPI)

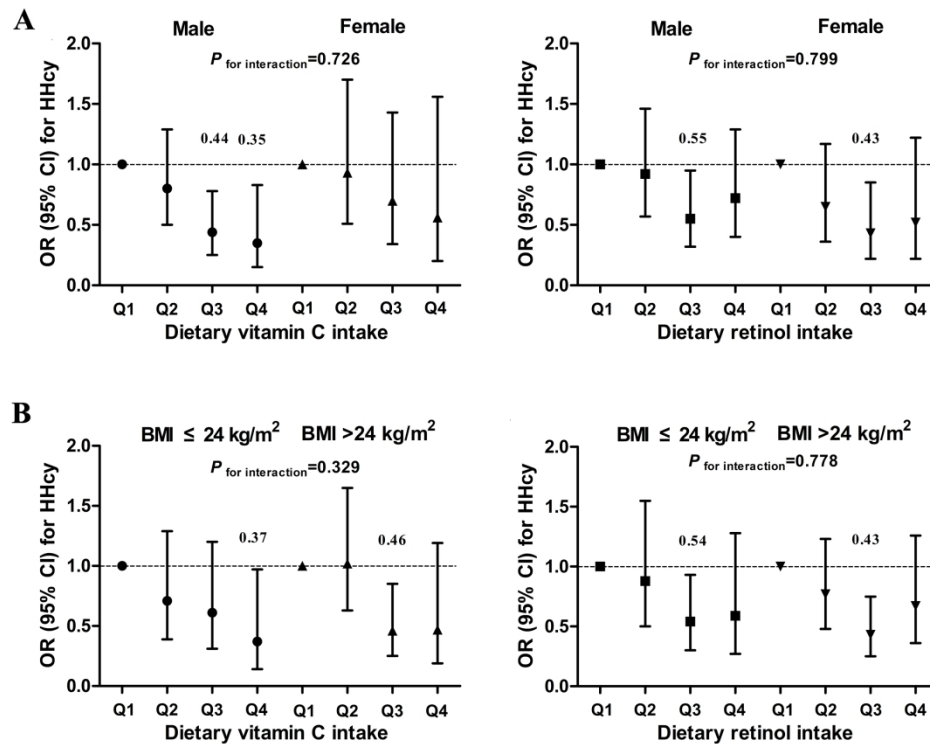


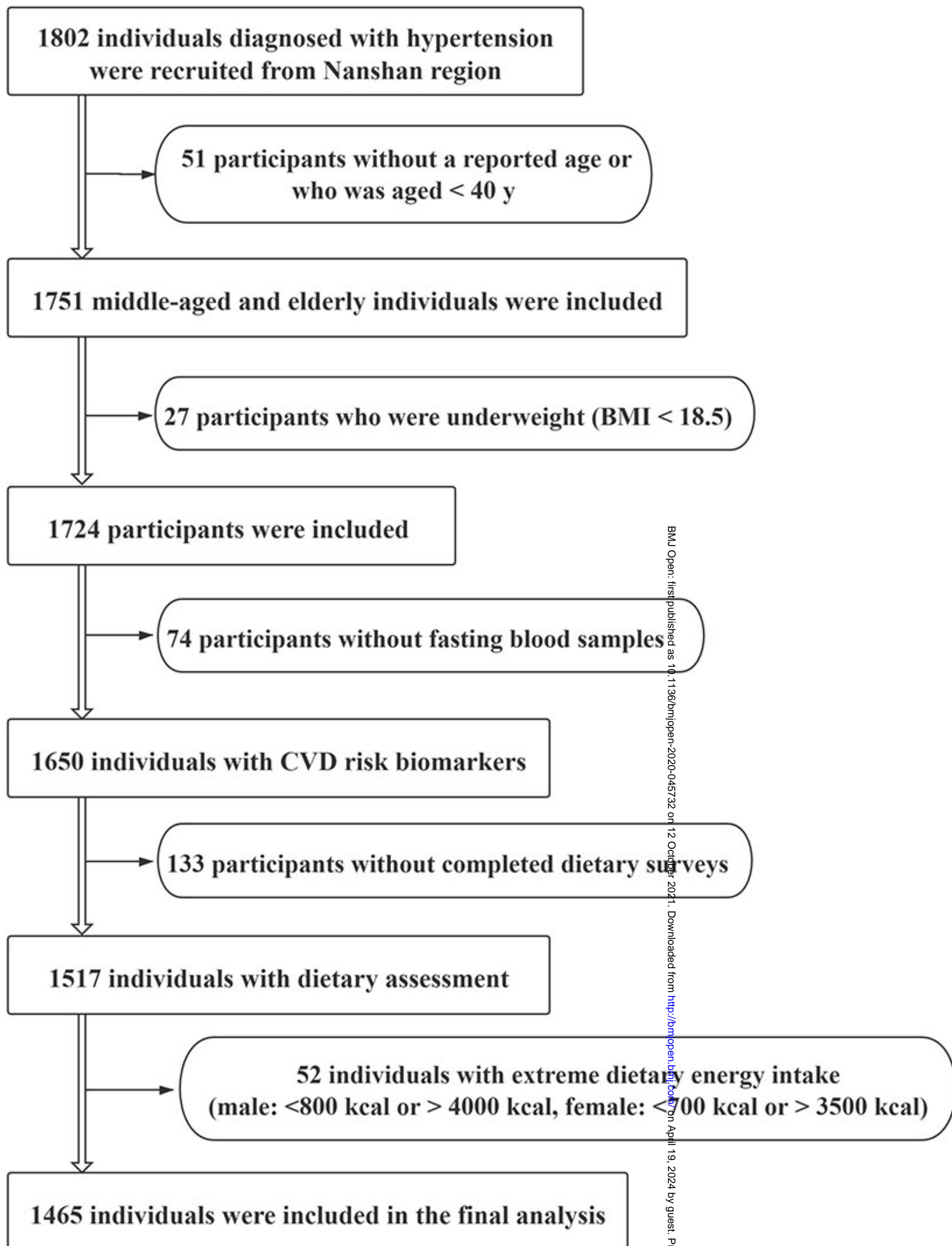
Fig. 2 ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to vitamin C, retinol intake in subgroups, stratified by sex (A), BMI (B). Adjusted for age, sex (except sex subgroup), BMI (except BMI subgroup), sedentary time (' < 3 h/d' as the reference), supplement use ('no' as the reference),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

127x96mm (600 x 600 DPI)

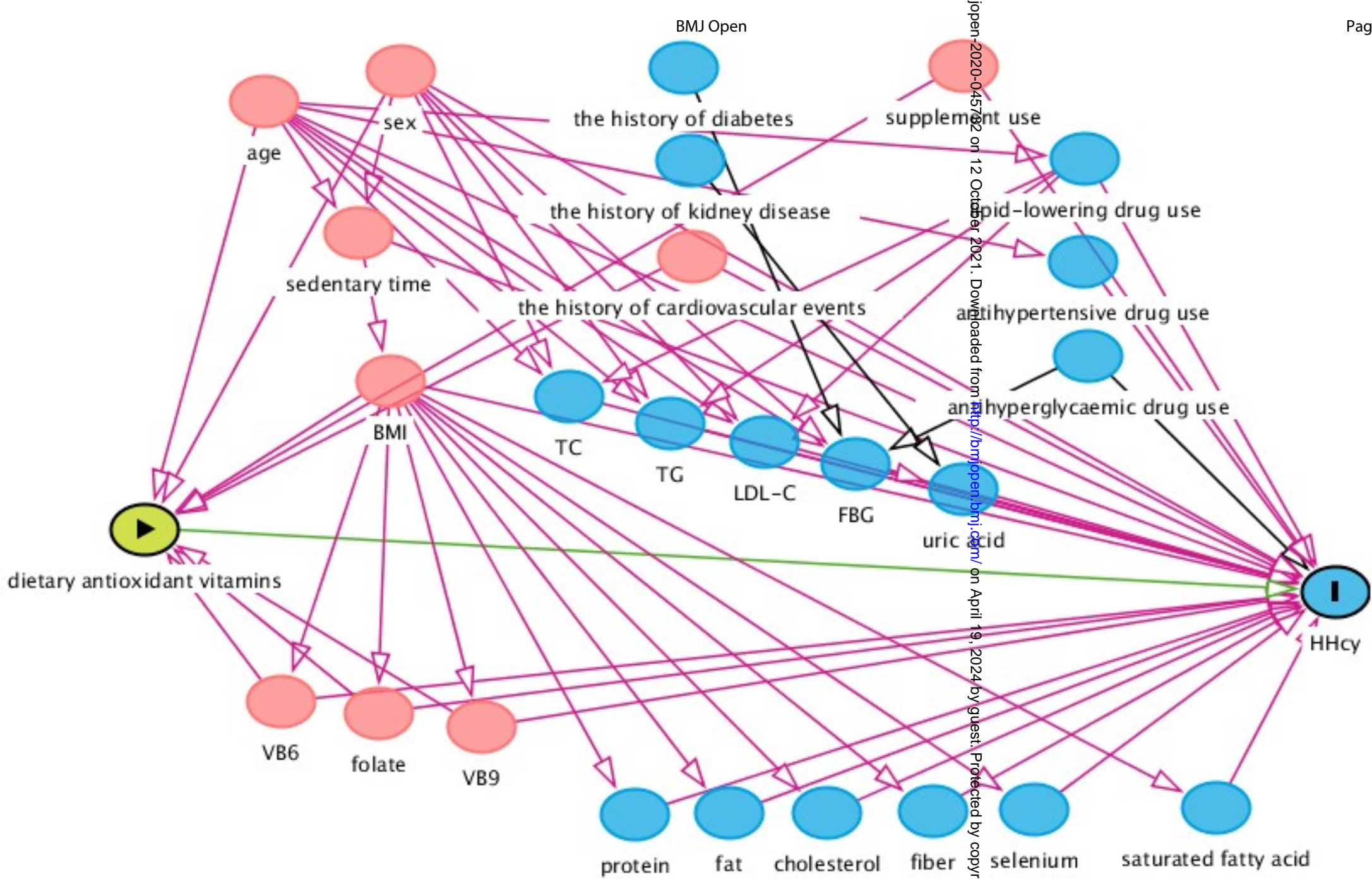
Supplementary Table 1 Sensitivity analyses of ORs (95% CIs) of antioxidant vitamins intake for hyperhomocysteinemia prevalence in multivariable-adjusted model among the patients with hypertension #

	Q1	Q2	Q3	Q4	<i>P</i> 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 B₆ and vitamin B₁₂.



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

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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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[#] Peng Xiaolin and Gao Qin contributed equally to this paper.

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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 $\mu\text{mol/L}$. 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.

61 Strengths and limitations of this study:

62 1. This study focused on the risk of hyperhomocysteinemia among middle-aged and
63 older adults with hypertension.

64 2. A linear inverse association between dietary VC intake and HHcy prevalence and
65 the threshold effect of retinol on HHcy was reported in this study.

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

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

71 Introduction

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

85 Increasing age, male sex, genetic factors, consumption of alcohol or tobacco, kidney
86 function and physical activity are some of the factors associated with tHcy levels (10).
87 Epidemiological studies and clinical trials have indicated that folate, vitamin B₁₂ and
88 vitamin B₆ status, well-known predictors of tHcy, are important for tHcy metabolism.
89 The latest meta-analysis demonstrated that a lower risk of stroke and overall
90 cardiovascular disease (CVD) with folic acid supplementation, which may partly

91 contribute to the decrease of tHcy levels (11).

92 Folic acid and vitamin B₁₂, which are involved in remethylation of homocysteine to
93 methionine by methionine synthase (MS), and vitamin B₆, which acts as a cofactor in
94 the transsulfuration of homocysteine to cysteine by cystathionine β-synthase (CBS)
95 (12). Folate, a key factor of tHcy metabolism, is very sensitive to free radicals (13-15).
96 In addition, MS and CBS were strongly influenced by oxidative stress, which may be
97 associated with dietary antioxidant vitamins and Hcy levels (16,17). We hypothesized
98 that antioxidant nutrients may regulate tHcy metabolism by influencing methionine-
99 homocysteine cycle. Plasma levels or dietary intake of vitamin C (VC), vitamin E (VE)
100 or β-carotene was inversely associated with tHcy levels, however, the findings were not
101 consistent (18-20). Of note, the association has never been investigated among the
102 hypertensive population.

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

106 **Methods**

107 *Study design and population*

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

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

121 mass index (BMI) < 18.5 kg/m²), as well as participants for whom fasting blood
122 samples (n = 74) and complete dietary surveys (n = 133) were not available. We also
123 excluded participants with an extreme dietary energy intake (male: < 800 kcal or > 4000
124 kcal, female: < 700 kcal or > 3500 kcal) (n = 52). Finally, 1465 participants were
125 included in the analysis (**Supplementary Figure 1**).

126 *Patient and public involvement*

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

131 *Dietary assessment*

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

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4 151 The intra-class correlation coefficients of two administrations of FFQ for nutrients of
5 152 VC, VE, carotenes, retinol, lutein were 0.395, 0.477, 0.355, 0.551 and 0.350, and were
6
7 153 all statistically significant.

9 154 ***Assessment of other covariates***

11 155 Participants' socio-demographic characteristics (e.g., age and sex), lifestyle factors
12 156 (e.g., sedentary time), history of chronic diseases, and medication and supplement use
13 157 status were collected. Sedentary time consisted of time spent watching TV and sitting,
14 158 which were combined into one variable with 2 categories, < 3 h/d and \geq 3 h/d, based on
15 159 a median sedentary time of 3 h/d. The history of cardiovascular events including
16 160 coronary heart disease, cerebral haemorrhage, cerebral thrombosis, and the history of
17 161 diabetes and kidney disease were recorded for each participant. The prescription use
18 162 status was classified into 2 groups (yes or no) corresponding to whether the participant
19 163 was taking any type or quantity of drugs, including antihypertensive drugs,
20 164 antihyperglycaemic drugs, lipid-lowering drugs or tHcy-lowering drugs. Supplement
21 165 use consisted of vitamin A, retinol, B vitamins, VC, VE and folic acid was included.

23 166 ***Anthropometric measurements***

25 167 Height and weight and waist circumference (WC) were measured by specialists. BMI
26 168 was calculated as weight (kg) / height (m²). Systolic blood pressure (SBP) and diastolic
27 169 blood pressure (DBP) were measured from the right arm of participants in a seated
28 170 position after a sufficient rest period using a mercury sphygmomanometer in the
29 171 morning. Blood pressure was measured manually and recorded as the average of three
30 172 measurements.

32 173 ***Laboratory tests and outcomes***

34 174 Morning fasting elbow vein blood samples were required to fast overnight (at least 10
35 175 hours) were collected from the participants at the CHSCs and transported under
36 176 refrigerated conditions to a clinical laboratory of the Nanshan Centre for Chronic
37 177 Disease Control within 2 hours. The blood specimens were collected in a 5-ml EDTA
38 178 vacuum tube. Blood samples were collected through deposition and centrifugation for
39 179 ten minutes at 3000 r/min at room temperature. The concentrations of plasma tHcy,
40 180 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 $\geq 15 \mu\text{mol/L}$.

Statistical analysis

Demographic characteristics were described by means \pm 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 B₆ and vitamin B₁₂, 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 5th, 35th, 65th and 95th percentiles of dietary antioxidant vitamins intake.

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

211 including sex (male or female) and BMI (<24 or ≥ 24 kg/m²), stratified analyses were
212 conducted by these potential factors and estimated *P* values for interaction terms.

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

215 Results

216 In this cross-sectional study, 1465 participants (male: 729, female: 736) were
217 included in the analysis, and the mean \pm SD of age and BMI was 62.0 ± 10.7 years, and
218 24.9 ± 3.5 kg/m². The tHcy levels of the participants was 14.63 ± 9.06 μ mol/L, and the
219 number (percentage) of HHcy was 469 (32.0%). The participants with HHcy were older
220 and more male, and have higher WC, SBP, tHcy, uric acid, creatinine, and higher
221 percentages of history of cardiovascular events (including coronary heart disease,
222 cerebral hemorrhage, cerebral thrombosis) and kidney disease, and have lower FPG
223 level (all $P < 0.05$) (**Table 1**).

224 The dietary intakes of nutrients were shown in **Table 2**. The median (IQR) of dietary
225 antioxidant vitamins intake were as follows: VC 153.5 (91.2, 240.9) mg/d, VE 25.0
226 (19.3, 31.9) mg/d, carotenes 3.3 (1.8, 5.6) mg/d, retinol 138.3 (78.0, 283.6) μ g/d, and
227 lutein 9.5 (5.2, 14.8) mg/d. Among the participants with HHcy, the intake of
228 carbohydrate was higher, and the intake of fruit, protein, fibre, retinol, folate, vitamin
229 B₁₂, saturated fatty acid, selenium, magnesium, zinc was lower, and the percentage of
230 supplement use was lower (all $P < 0.05$).

231 The association between dietary antioxidant vitamins intake and tHcy levels are
232 shown in **Table 3**. The inverse association between VC intake and tHcy concentration
233 after ln-transformed in the fully adjusted model, and the β (95% CIs) was -0.050 (-
234 0.084, -0.016), which was consistent with the logistic regression as categorical
235 variables. In Model II, the significant association was found in the third and highest
236 quartile of VC intake, and the ORs (95% CIs) were 0.49 (0.33, 0.74) and 0.40 (0.22,
237 0.74) ($P_{\text{for trend}} = 0.001$). The retinol intake was also inversely associated with tHcy, as
238 the β (95% CIs) was -0.021 (-0.041, -0.002). After adjusting for age, sex and BMI, the
239 ORs (95% CIs) for HHcy prevalence across quartiles of retinol intake were 1.00, 0.84
240 (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

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

247 After fully adjusting for the potential confounders, the association between dietary
248 antioxidant vitamins intake and the prevalence of HHcy were shown in **Figure.1**. From
249 the cubic splines, we noted that the linear inverse trend of VC intake and HHcy
250 prevalence (P for overall association=0.016, P for nonlinearity=0.055) and the L-
251 shaped relationship of retinol intake and HHcy prevalence (P for overall
252 association=0.011, P for nonlinearity=0.020), which were consistent with the results of
253 logistic regression analyses. The threshold analysis showed that the ORs (95% CIs) of
254 HHcy was 0.995 (0.991, 0.995) ($P=0.005$) when retinol intake was lower than 147.2
255 $\mu\text{g/d}$ and 1.000 (1.000, 1.001) ($P=0.094$) when retinol intake was more than 147.2 $\mu\text{g/d}$,
256 and the P value of log-likelihood ratio was 0.003. The non-association between
257 carotenes, lutein and VE and HHcy was not shown.

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

262 Discussion

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

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

271 to show the effect of folate and vitamins B₁₂ and B₆ supplement on lowering the levels
272 of tHcy, however, the negative results were found in most studies (26). The potential
273 reason may be correlated with the harmful effect of unmetabolized excessive folate or
274 the cyanide and thiocyanate from excessive cyanocobalamin. In China, where folate
275 fortification has not been implemented, folic acid significantly reduced the risk of
276 stroke was observed from the China Stroke Primary Prevention Trial (CSPPT) (27).
277 Thus, appropriate B vitamins therapy is of great importance for lowering tHcy levels in
278 stroke prevention.

279 In the past decades, several researches reported the potential association between
280 plasma levels or intake of different antioxidant vitamins and tHcy levels (18, 19, 28-
281 30). In 1999, Brude IR *et al.* observed the inverse association between plasma tHcy
282 concentration and dietary intake of vegetables, vitamin C and β -carotene from 41
283 participants (28). In addition, dietary intake of retinol equivalents, β -carotene and VC
284 were inversely correlated with plasma tHcy levels, after adjustment for dietary B-
285 vitamins, but not after additional adjustment for plasma folate and vitamin B₁₂ (19).
286 What's more, the study focused on the effect of antioxidant vitamins on the plasma
287 tHcy levels in a free-living elderly population found that plasma VC, rather than the
288 intake and supplementation of VC, showed a negative association with tHcy in simple
289 regression analysis, and also found that the plasma levels, as well as the intake and
290 supplementation of vitamin E, and β -carotene were not associated with tHcy (18).
291 Similarly, the cross-sectional NHANES 1999–2002 study found that dietary VC and
292 VE intake were associated with a lower prevalence of elevated blood tHcy
293 concentration, whereas no association between dietary carotenes intake and tHcy was
294 detected (29). In addition, Rajesh Ullegaddi *et al.* found that significant reductions in
295 plasma homocysteine in the group with antioxidant treatment (vitamins C and E)
296 combination with B-vitamin, compared with the group with B-vitamin alone (30),
297 which support the importance of antioxidant vitamins on tHcy metabolism.

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

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4 301 activates the folate-mediated one-carbon cycle in C2C12 myoblasts (32). Thus, VC has
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6 302 been explored as an attractive factor to increase circulating levels of folic acid and to
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8 303 reduce Hcy levels.

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10 304 We found an L-shaped association between dietary retinol intake and high tHcy
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12 305 prevalence, which meant that if the retinol intake was low, the risk of HHcy was
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14 306 decreased as retinol intake increased, but the risk was not changed when retinol intake
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16 307 reached certain level, which was more than 147.2 $\mu\text{g}/\text{d}$ among the participants in this
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18 308 study. The dietary intake of retinol equivalents was inversely correlated with plasma
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20 309 tHcy levels after adjustment for dietary B-vitamins (19). Retinol, a preformed vitamin
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22 310 A, plays an important role in vision, cellular differentiation, and proliferation, as well
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24 311 as the immune system regulation. In addition, there is increasing evidence indicates that
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26 312 retinol seems to inhibit thrombosis (33) and inflammation effects (34), which indicates
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28 313 retinol is emerging as a factor of interest to CVD. Brazionis L *et al.* reported that plasma
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30 314 retinol was a novel marker for CVD mortality in Australian adults, with an inverse
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32 315 association between plasma retinol in the middle tertile and 5-year CVD mortality (35).
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34 316 Similarly, a strong association between low retinol and the risk of sudden cardiac death
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36 317 was examined (36). In addition, a nested case-control study showed a significant
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38 318 inverse association between plasma retinol and the risk of first stroke among Chinese
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40 319 hypertensive adults from the CSPPT (37), which may due to relatively low baseline
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42 320 retinol concentrations (median: 67.5 $\mu\text{g}/\text{dL}$). Besides, the interaction of retinol and tHcy
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44 321 on CVD risk was also reported. Yu Y *et al.* (37) showed the inverse effect between
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46 322 plasma retinol and first stroke was stronger among the participants whose tHcy < 10
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48 323 $\mu\text{mol}/\text{L}$ than whose tHcy ≥ 10 $\mu\text{mol}/\text{L}$. Olsen T *et al.* (38) found that the plasma tHcy
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50 324 was associated with acute myocardial infarction only in the upper Vit-A tertile, and the
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52 325 potential mechanisms may include inflammation and lipid metabolism, which may be
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54 326 partly interpreted with the high intake of retinol (1576 μg RAE/d).

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56 327 In addition, the dietary source of retinol may contribute to the non-significant effect
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58 328 of retinol in the highest quartile in the fully adjusted model, as it is present in animal-
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60 329 based foods, particularly in liver and whole milk. In this study, we found the TG level
330 was gradually increased with the increase of retinol (data not shown). High retinol

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

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

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

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

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

360 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, B₁₂, B₆, 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 B₁₂. 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)	non-HHcy (n=996)	<i>P</i>
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)	388.7 (252.5, 577.1)	0.510
Fruit (g)	79.2 (39.7, 139.5)	75.4 (33.2, 127.5)	81.4 (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)	236.8 (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)	151.0 (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)	176.7 (112.8-266.9)	0.009

continued on next page

Table 2 (continued)

	Total	HHcy (n=469)	non-HHcy (n=996)	<i>P</i>
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)	221.3 (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 level among the middle-aged and older hypertensive participants

	tHcy	Q1	Q2	Q3	Q4	<i>P</i> for trend
Vitamin C (mg/d)		< 91.2	91.2-153.5	153.6-240.9	\geq 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	\geq 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	\geq 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	<i>P</i> 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).

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4 540 Figure Legends:

5 541 **Fig. 1** Restricted cubic spline analyses illustrating the shapes of multivariable
6 association between dietary vitamin C intake (A), or dietary retinol intake (B) and
7 HHcy. Adjusted for age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as
8 the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin
9 B₆ and vitamin B₁₂, the history of cardiovascular events ('no' as the reference).
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12 545
13 546 **Fig. 2** ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to
14 vitamin C, retinol intake in subgroups, stratified by sex (A), BMI (B). Adjusted for age,
15 sex (except sex subgroup), BMI (except BMI subgroup), sedentary time ('< 3 h/d' as
16 the reference), supplement use ('no' as the reference), dietary intake of folate, vitamin
17 B₆ and vitamin B₁₂, the history of cardiovascular events ('no' as the reference).
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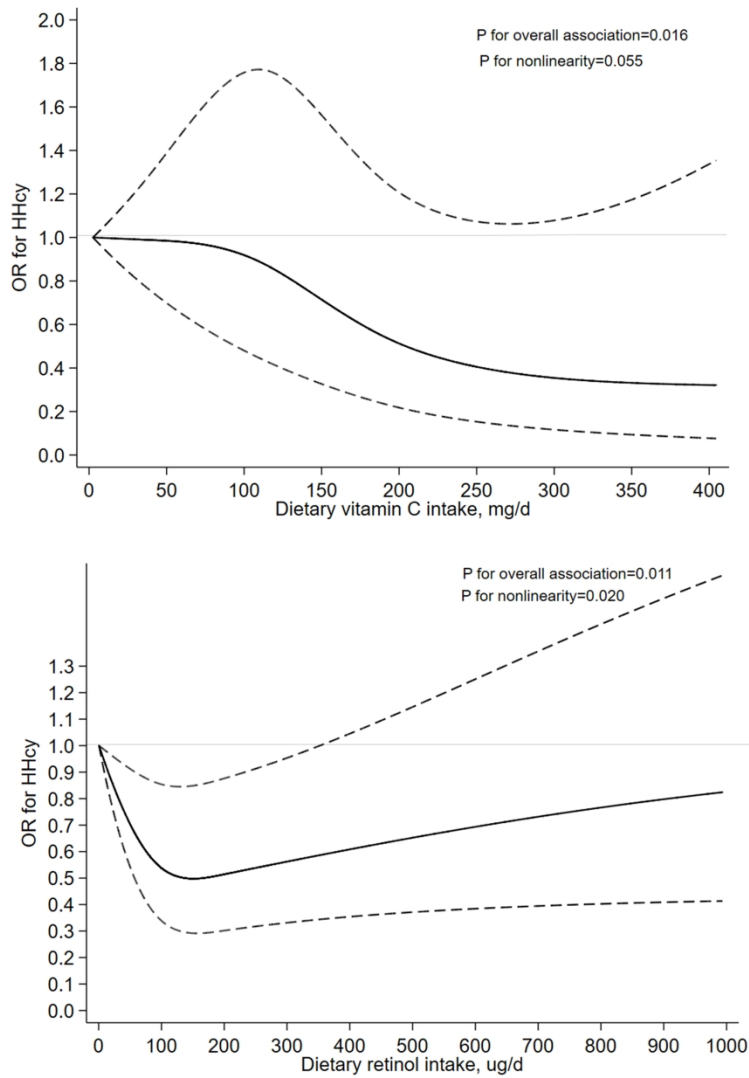


Fig 1. Restricted cubic spline analyses illustrating the shapes of multivariable association between dietary vitamin C intake (A), or dietary retinol intake (B) and HHcy. Adjusted for age, sex ('male' as the reference), BMI, sedentary time ('< 3 h/d' as the reference), supplement use ('no' as the reference),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

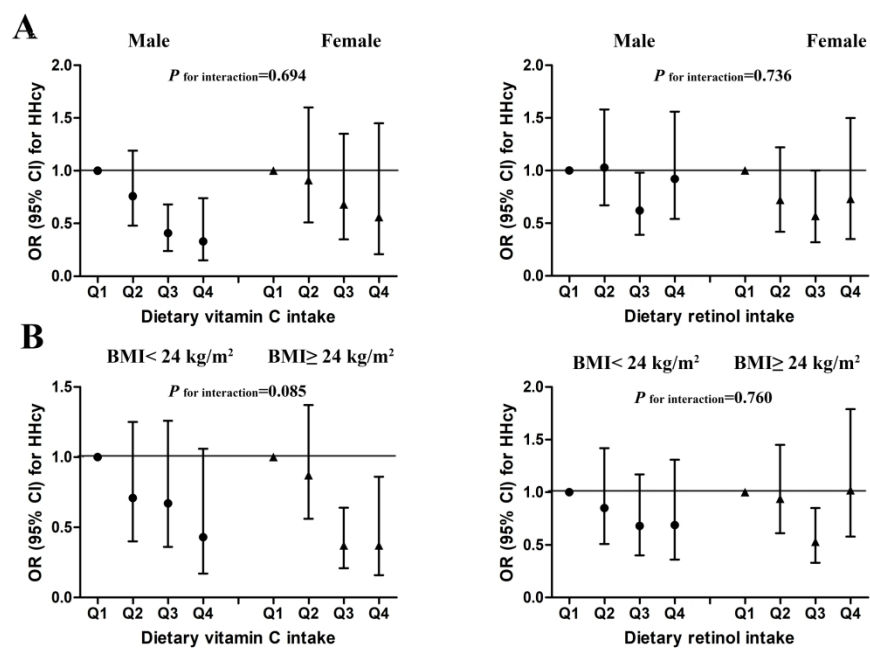


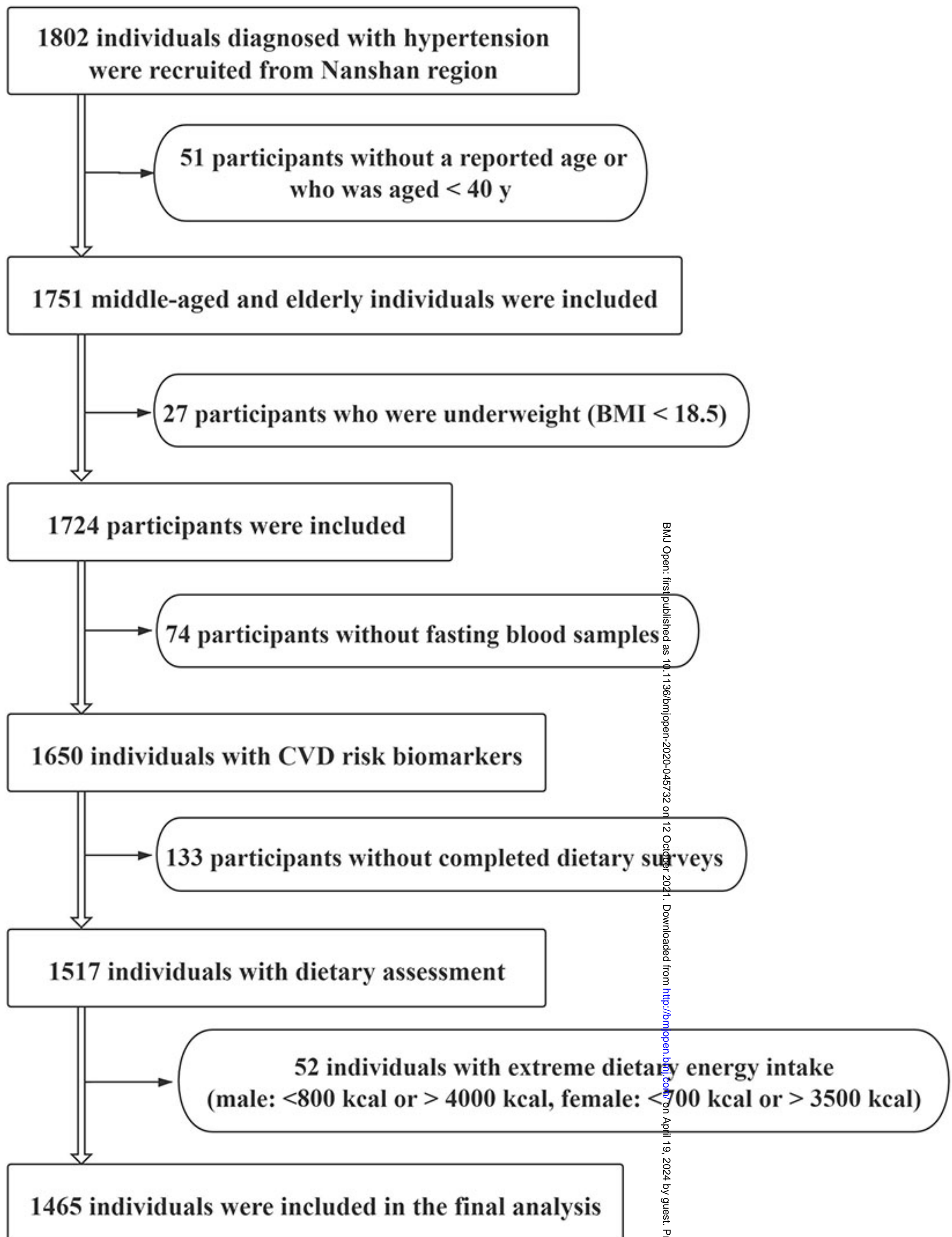
Fig 2. ORs and 95% CIs for hyperhomocysteinemia (HHcy) prevalence according to vitamin C, retinol intake in subgroups, stratified by sex (A), BMI (B). Adjusted for age, sex (except sex subgroup), BMI (except BMI subgroup), sedentary time (< 3 h/d' as the reference), supplement use ('no' as the reference),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

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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 #

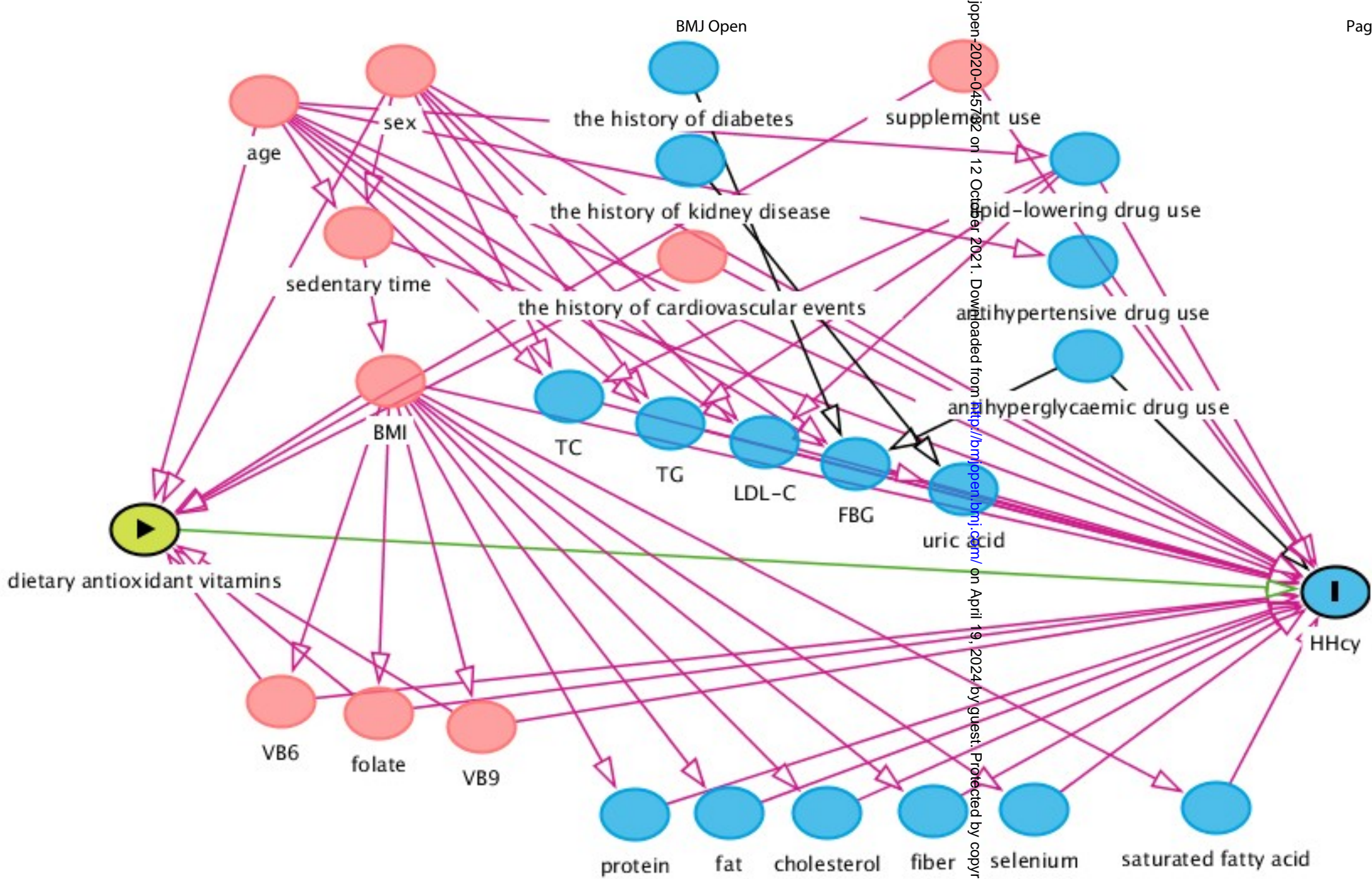
	Q1	Q2	Q3	Q4	<i>P</i> for trend
Without cardiovascular events (n=1245)					
Vitamin C	1.00 (Ref.)	0.92 (0.63, 1.35)	0.54 (0.35, 0.84)	0.41 (0.21, 0.80)	0.003
Vitamin E	1.00 (Ref.)	1.14 (0.79, 1.64)	0.95 (0.66, 1.39)	0.90 (0.62, 1.31)	0.425
Carotenes	1.00 (Ref.)	1.25 (0.85, 1.84)	0.67 (0.43, 1.04)	0.77 (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 B₆ and vitamin B₁₂.



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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

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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.