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Original article

# Plasma 25-hydroxyvitamin D concentrations and risk of incident cancer in adults with hypertension: A nested case–control study

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SUMMARY

*Background & aims:* Evidence from epidemiologic studies on the association of circulating 25-hydroxyvitamin D [25(OH)D] concentrations with the incident risk of cancer has been inconsistent. We aimed to investigate the prospective relationship of baseline plasma 25(OH)D concentrations with the risk of cancer, and to examine possible effect modifiers.

*Methods:* We employed a nested case–control study design, including 231 patients with incident cancer during a median 4.5 years of follow up, and 231 matched controls from the China Stroke Primary Prevention Trial (CSPPT).

*Results:* The prevalence of plasma 25(OH)D <15, <20 and <30 ng/mL was 23.6%, 47.4% and 85.5%, respectively. Overall, there was an inverse relation between risk of cancer and plasma 25(OH)D. The Odds ratios (95% CI) for participants in the second (15.1 to <20.6 ng/mL), third (20.6 to <26.4 ng/mL) and fourth quartiles (≥26.4 ng/mL) were 0.45 (95% CI: 0.25–0.80), 0.53 (95% CI: 0.27–1.06) and 0.55 (95% CI: 0.27–1.10), respectively, compared with those in quartile 1. Conversely, low 25(OH)D (<15.1 ng/mL) concentrations were associated with increased risk of cancer (OR, 2.08; 95% CI: 1.20–3.59) compared to higher concentrations. These associations were consistent across subtypes of cancer. Several potential effect modifiers were identified, including plasma vitamin E concentrations and alcohol intake.

*Conclusions:* Low plasma 25(OH)D concentrations (<15.1 ng/mL) were associated with increased total cancer risk among Chinese hypertensive adults, compared to higher 25(OH)D concentrations. This finding and the possible effect modifiers warrant additional investigation.



*Abbreviations:* BMI, body mass index; CI, confidence interval; CSPPT, China stroke primary prevention trial; DBP, diastolic blood pressure; OR, odds ratio; SBP, systolic blood pressure; 1,25[OH]2D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

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## 1. Introduction

Cancer is a substantial clinical and public health problem in China and globally [1,2]. An estimated 14.1 million new cancer cases and 8.2 million cancer deaths occurred worldwide in 2012 [3], and approximately 4.3 million new cancer cases and 2.8 million cancer deaths occurred in China in 2015 [4]. There is an urgent need to identify important and modifiable risk factors to halt and reverse the rising trend of cancer.

In addition to well-known risk factors associated with cancer (i.e., age, tobacco use, infection, overweight and physical inactivity), there is growing interest in the role of vitamin D in cancer prevention, given its many anti-cancer properties, such as anti-inflammation, anti-proliferation, inhibition of angiogenesis and stimulation of differentiation [5–7].

Vitamin D is a group of fat-soluble secosteroids, mainly including vitamin $D_2$ (ergocalciferol) and vitamin $D_3$ (cholecalciferol). Vitamin D can be obtained via skin exposure to sunlight and from the diet or supplements. Circulating 25(OH)D is considered the more sensitive marker of vitamin D status because it has a longer half-life in the circulation (greater than 3 weeks) than $1,25(OH)_2D$ (a few hours) [8].

In the past few years, a rapidly increasing number of epidemiologic studies have assessed the association of circulating 25(OH)D concentrations with the risk of cancer, however, results have been inconsistent. Several ecological studies in Chinese and Japanese populations have reported that increased exposure to solar radiation may reduce incident risk of cancers or improve the outcome of cancers, supporting the hypothesis that vitamin D synthesized photochemically in the skin may have anti-carcinogenic effects [9,10]. Many case–control studies have suggested a significant inverse association between circulating vitamin D concentrations and cancer risk [11–13], whereas subsequent prospective or randomized trials have reported inconclusive results [14–16]. The primary reason for the discrepancy in results between case–control and prospective studies is that the former could not tease out temporal relationship between exposure and outcome; and serum 25OHD concentration changes with respect to time [17,18]. Besides differences in study design, other reasons may include differences in geographic locations, definition of endpoints (total *vs.* cancer subtypes), population characteristics, and median and range of vitamin D concentrations in the study populations. Of note, previous vitamin D clinical trials were based on vitamin D dose rather than serum 25OHD concentration [19,20], as such, they did not take into account of individual variations in genetic background, baseline vitamin D status, absorption, and metabolism. Furthermore, the possible interactions of vitamin D with traditional risk factors or other vitamins have not been fully considered.

Our current study aimed to evaluate the relationship of baseline plasma 25(OH) vitamin D concentrations with the incident risk of total cancer (and subtypes), and to examine any possible effect modifiers using a nested case–control design. Subjects were participants in the China Stroke Primary Prevention Trial (CSPPT), which consisted of over 20,000 Chinese hypertensive adults [21], where total cancer and specific cancer types were obtained as a pre-specified endpoint [22].

## 2. Materials and methods

### 2.1. Study population

The methods and major results of the CSPPT have been reported elsewhere [22]. Briefly, the CSPPT was a multi-community, randomized, double-blind, controlled trial conducted from May 19, 2008 to August 24, 2013 in 32 communities in China. Eligible participants were men and women aged 45–75 years with hypertension, defined as seated resting systolic blood pressure (SBP) $\geq$ 140 mmHg or diastolic blood pressure (DBP) $\geq$ 90 mmHg at both the screening and recruitment visit or, who were taking antihypertensive medication. The major exclusion criteria included history of physician-diagnosed stroke, myocardial infarction (MI), heart failure, post-coronary revascularization, and/or congenital heart disease.

The CSPPT was approved by the Ethics Committee of the Institute of Biomedicine, Anhui Medical University, Hefei, China (FWA assurance number: FWA00001263) and registered with ClinicalTrials.gov, NCT00794885. All participants gave prior written informed consent.

### 2.2. Intervention and follow-up

A total of 20,702 eligible participants were randomly assigned, in a 1:1 ratio, to one of two treatment groups: a daily oral dose of one tablet containing 10 mg enalapril and 0.8 mg folic acid (the enalapril-folic acid group), or a daily oral dose of one tablet containing 10 mg enalapril only (the enalapril group). Participants were followed-up every three months. At each visit, trained study personnel measured blood pressure and pulse, and recorded the number of pills taken between visits, concomitant medication use, and any adverse events for all participants.

### 2.3. Outcomes

Cancer incidence, a pre-specified endpoint of the CSPPT, was the main outcome in this analysis. Cancer could be diagnosed based on either positive pathologic findings or specific clinical manifestations. Acceptable evidence for pathologic findings included original or photocopied pathological reports, and original or photocopied medical records from hospitals in which pathological results were cited. When pathological data were not available, cases were independently reviewed by two oncologists. Cancer was diagnosed only when both physicians made the same clinical diagnosis based on clinical manifestations and examinations.

All cancer events were reviewed and adjudicated by an independent Endpoint Adjudication Committee, whose members were unaware of study-group assignments.

### 2.4. Nested case–control study

During a median treatment duration of 4.5 years (IQR: 4.2–4.7 years), cancer occurred in 116 participants (1.12%) in the enalapril-folic acid group as compared to 116 participants (1.12%) in the enalapril group (HR, 1.00; 95% CI, 0.77–1.29; $P > 0.99$) [22].

Using data from the CSPPT, we established a nested case–control study of 232 incident cancer cases and 232 matched controls within this cohort. Controls were randomly chosen from the baseline CSPPT participants who did not develop cancer during the follow-up, and were matched by age (±1 year), sex, treatment group and study site with cases on a 1:1 ratio. For the current analysis, 231 incident cases and 231 matched controls were included (Fig. 1). The study was approved by the Ethics Committee of the Institute of Biomedicine, Anhui Medical University, Hefei, China.

### 2.5. Laboratory assays

Serum folate and vitamin B12 were measured by a commercial laboratory using a chemiluminescent immunoassay (New Industrial, Shenzhen, China). Serum fasting lipids and glucose

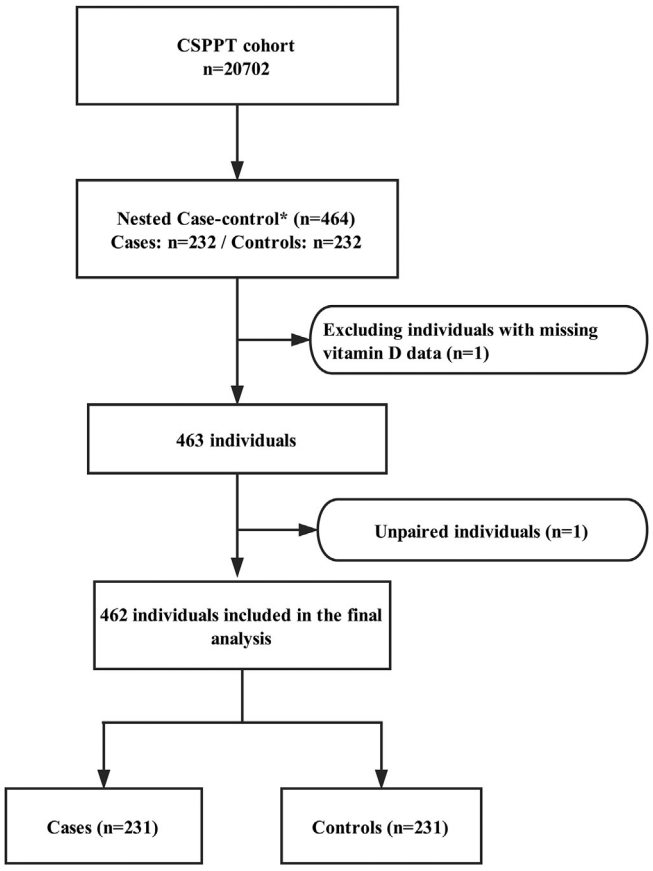


**Fig. 1.** Flow chart of the study participants using a nested case–control design. 232 controls were individually matched to 232 cases on age (within 1 year), sex, treatment group, and study site.

concentrations at baseline were measured using automatic clinical analyzers (Beckman Coulter) at the core laboratory of the National Clinical Research Center for Kidney Disease, Nanfang Hospital, Guangzhou, China. Plasma vitamin A, vitamin E, vitamin K, 25(OH)$D_3$ and 25(OH)$D_2$ were measured by liquid chromatography with tandem quadrupole mass spectrometry (LC-MS/MS), and plasma calcium was measured by inductively coupled plasma mass spectrometry (ICP-MS) in a commercial lab (Beijing DIAN Medical Laboratory, China). Total 25(OH)D was used in all analyses and calculated as the sum of 25(OH)$D_3$ and 25(OH)$D_2$.

### 2.6. Statistical analyses

Differences in baseline characteristics between cases and controls were compared using chi-square tests for categorical variables and generalized paired t tests for continuous variables.

Odds ratios of cancer in relation to plasma concentrations of 25(OH)D were calculated using conditional logistic regression models. Plasma 25(OH)D concentrations were categorized into quartiles based on the distribution. Potential confounders included in the logistic regression models, other than the matching criteria, were body mass index (BMI), smoking status, alcohol consumption, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting glucose, total cholesterol, triglycerides, calcium, folate, vitamin B12, vitamin A, vitamin E, vitamin K, and season of blood collection, as well as time-averaged SBP and DBP during the treatment period.

In further exploratory analyses, potential effect modifications of the association between plasma 25(OH)D concentrations and cancer risk were assessed for the variables: age (<60 *vs.* ≥60 years), sex, treatment group (enalapril *vs.* enalapril-folic acid treatment), current smoking (yes *vs.* no), current alcohol intake (yes *vs.* no), folate (<8.5 [median] *vs.* ≥8.5 ng/mL), vitamin B12 (<383.1 [median] *vs.* ≥383.1 ng/mL), vitamin A (<0.7[median] *vs.* ≥0.7 μg/L), vitamin E (<8.9 [median] *vs.* ≥8.9 ng/mL), calcium (<96.7 [median] *vs.* ≥96.7 mg/L), and season at time of blood collection (spring or winter *vs.* summer or autumn). Potential interactions were examined by including the interaction terms into those logistic regression models with the greatest number of confounding variables.

A two tailed $P < 0.05$ was considered statistically significant in all analyses. R software, version 3.2.5 (http://www.R-project.org/) was used for all statistical analyses.

## 3. Results

### 3.1. Study participants and baseline characteristics

This study included 231 subjects with incident cancer, diagnosed during the period from the time of blood collection until the end of follow-up, and 231 matched controls within the CSPPT cohort (Fig. 1). Among the 231 incident cancer cases, 129 were gastrointestinal cancer (53 esophageal, 43 gastric, 19 colorectal and 14 other sites) and 102 were non-gastrointestinal cancer (27 breast, 26 lungs and 49 other sites) (Supplemental Table 1). The mean age at blood collection was 61.8 years (SD, 7.0). The mean plasma 25 (OH) D was approximately 21.5 ng/mL (SD, 9.1). The prevalence of plasma 25 (OH) D <15, ≤20 and ≤30 ng/mL was 23.6%, 47.4% and 85.5%, respectively.

There were no major differences in characteristics between cases and controls, although cases had slightly higher mean SBP levels during the treatment period, and were more likely to smoke than controls (Table 1). Patients with lower plasma 25(OH)D concentrations tended to be female, had higher BMI, calcium, baseline SBP levels, lower folate, vitamin A, vitamin E concentrations, and were less likely to consume alcohol and have their blood sample collected in spring or winter (Supplemental Tables 2 and 3). Similar results were found when plotting serum 25OHD *vs.* Julian day of the year for both cases and controls (Supplemental Fig. 2).

More importantly, as shown in Supplemental Fig. 1, compared to the control subjects, the distribution of the plasma 25(OH)D clearly shifted towards the left tail in the case subjects.

### 3.2. Association between plasma 25 (OH) D concentrations and risk of cancer (total and subtypes)

The median length of follow up was 4.5 years (interquartile range, 4.2–4.7 years). The association between plasma 25 (OH) D concentrations and risk of cancer is presented in Fig. 2. Overall, the risk of cancer decreased with the increase of plasma 25 (OH) D.

Consistently, when plasma 25(OH) D concentrations were assessed as quartiles, a significantly lower risk of total cancer (OR, 0.48; 95% CI: 0.28–0.83) was found in participants in quartiles 2–4 (≥15.1 ng/mL) compared with participants in quartile 1 (<15.1 ng/mL). The relative risks (95% CI) for participants in the second (15.1 to <20.6 ng/mL), third (20.6 to <26.4 ng/mL) and fourth quartiles (≥26.4 ng/mL) were 0.45 (95% CI: 0.25–0.80), 0.53 (95% CI: 0.27–1.06) and 0.55 (95% CI: 0.27–1.10), respectively, when compared with those in quartile 1 (Table 2).

Conversely, a significantly higher risk of cancer (OR, 2.08; 95% CI: 1.20–3.59) was found in participants in quartile 1 (<15.1 ng/mL) compared with participants in quartiles 2–4 (≥15.1 ng/mL) (Table 2).

**Table 1**
Characteristics of cases and control subjects.[a]

| Characteristics | Cases ($n$ = 231) | Controls ($n$ = 231) | $P$ value |
|---|---|---|---|
| Age, y | 61.8 (7.0) | 61.8 (7.0) | 0.31 |
| Male, No. (%) | 121 (52.4) | 121 (52.4) | 1.00 |
| Body mass index, kg/m$^2$ | 24.1 (3.6) | 24.4 (3.8) | 0.37 |
| Current smoking, No. (%) | 91 (39.4) | 70 (30.4) | 0.04 |
| Current alcohol drinking, No. (%) | 66 (28.6) | 67 (29.1) | 0.90 |
| Blood pressure, mmHg | | | |
| Baseline SBP | 163.9 (18.8) | 164.9 (17.3) | 0.52 |
| Baseline DBP | 92.5 (11.6) | 93.6 (11.9) | 0.28 |
| Mean SBP during treatment period | 140.5 (12.1) | 138.1 (9.7) | 0.02 |
| Mean DBP during treatment period | 83.1 (9.0) | 82.2 (7.8) | 0.20 |
| Treatment group, No. (%) | | | 1.00 |
| Enalapril | 115 (49.8) | 115 (49.8) | |
| Enalapril-folic acid | 116 (50.2) | 116 (50.2) | |
| Laboratory results | | | |
| TC, mmol/L | 5.4 (1.2) | 5.4 (1.2) | 0.82 |
| TG, mmol/L | 1.6 (0.9) | 1.6 (0.7) | 0.55 |
| Glucose, mmol/L | 5.6 (1.7) | 5.8 (1.9) | 0.14 |
| 25(OH)D, ng/mL | 21.2 (10.1) | 21.7 (8.0) | 0.50 |
| Folate, ng/mL | 8.9 (3.8) | 9.1 (4.4) | 0.58 |
| Vitamin B12, pg/mL | 410.9 (131.7) | 426.0 (160.7) | 0.31 |
| Vitamin A, μg/mL | 0.7 (0.3) | 0.7 (0.3) | 0.29 |
| Vitamin E, μg/mL | 9.5 (3.7) | 9.4 (3.3) | 0.71 |
| Vitamin K, ng/mL | 0.7 (1.0) | 0.7 (0.7) | 0.52 |
| Calcium, mg/L | 97.1 (8.6) | 97.8 (8.7) | 0.27 |
| Season of blood collection, No. (%) | | | 0.96 |
| Spring | 56 (24.2) | 52 (22.5) | |
| Summer | 67 (29.0) | 69 (29.9) | |
| Autumn | 66 (28.6) | 65 (28.1) | |
| Winter | 42 (18.2) | 45 (19.5) | |

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; 25(OH)D, 25-hydroxyvitamin D.
[a] For continuous variables, values are presented as means (SD).

Furthermore, adjusting for the date of blood draw (Julian day of the year) did not significantly alter the odds ratios (Supplemental Table 4).

Similar trends were observed for gastrointestinal or non-gastrointestinal cancers. A higher risk of gastrointestinal cancer (OR, 1.47; 95% CI: 0.65–3.31) and non-gastrointestinal cancer (OR, 3.64; 95% CI: 1.31–10.13) was found in participants in quartile 1 compared with those in quartiles 2–4 (Supplemental Table 5).

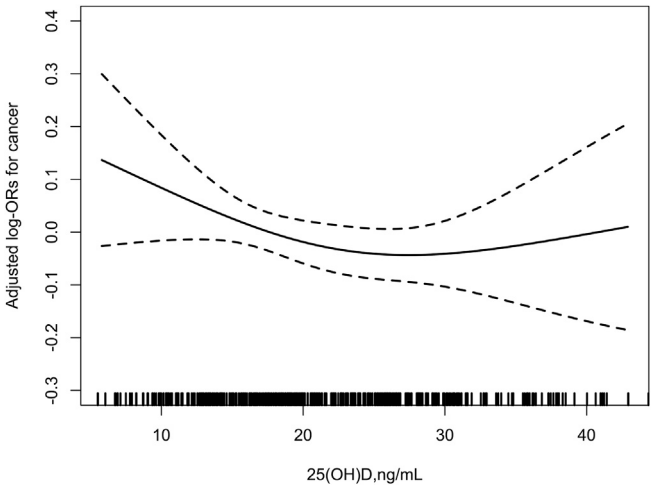


**Fig. 2.** The association between baseline plasma 25-Hydroxyvitamin D and incident risk of total cancer. In addition to the matching factors (age, sex, treatment group, center), the splines also adjusted for BMI, smoking status, alcohol consumption, baseline SBP and DBP, time-averaged SBP and DBP during treatment, baseline fasting blood glucose, total cholesterol, triglycerides, calcium concentrations, season of blood collection, folate, vitamin B12, vitamin A, vitamin E and vitamin K.

### 3.3. Stratified analyses by potential effect modifiers

Stratified analyses were performed to assess the association between plasma 25(OH)D concentrations and total cancer risk in various subgroups (Fig. 3). A significantly stronger inverse association between plasma 25(OH)D (quartiles 2–4, ≥15.1 *vs.* quartile 1, <15.1 ng/mL) and cancer risk was observed in non-alcohol drinkers (*vs.* drinkers, $P$ for interaction = 0.05) and in participants with higher vitamin E concentrations (≥8.9 *vs.* <8.9 ng/mL, $P$ for interaction = 0.002).

However, none of the other variables, including age (<60 *vs.* ≥60 years, $P$ for interaction = 0.94), sex ($P$ for interaction = 0.97), current smoking (yes *vs.* no, $P$ for interaction = 0.48), treatment group (enalapril *vs.* enalapril folic acid treatment, $P$ for interaction = 0.69), season of blood collection (spring or winter *vs.* summer or autumn, $P$ for interaction = 0.10), vitamin B12 (<383.1 *vs.* ≥383.1 ng/mL, $P$ for interaction = 0.67), folate (≥8.5 *vs.* <8.5 ng/mL, $P$ for interaction = 0.18), vitamin A (<0.7 *vs.* ≥0.7 μg/L (<$P$ for interaction = 0.81)), or calcium (<96.7 *vs.* ≥96.7 mg/L, $P$ for interaction = 0.65), obviously modified the association between plasma 25(OH)D (quartiles 2–4, ≥15.1, *vs.* quartile 1, <15.1 ng/mL) and total cancer risk.

## 4. Discussion

In this CSPPT population with relatively low median vitamin D and large variability across individuals, we found that a higher plasma 25(OH)D concentration (≥15.1 ng/mL, quartiles 2–4) was associated with a lower risk of total cancer in comparison to lower concentrations (<15.1 ng/mL, quartile 1). Conversely, low plasma 25(OH)D concentrations (<15.1 ng/mL) were associated with an increased risk of total cancer among Chinese hypertensive adults, compared to higher 25(OH)D concentrations. These results

**Table 2**
The association between baseline plasma 25-Hydroxyvitamin D and incident risk of total cancer.[a]

| 25 (OH) D, ng/mL | Cases/controls | Model 1 | | Model 2 | |
|---|---|---|---|---|---|
| | | OR (95% CI) | *P* | OR (95% CI) | *P* |
| Quartiles | | | | | |
| Q1 (<15.1) | 70/46 | *Ref* | | *Ref* | |
| Q2 (15.1 to <20.6) | 49/66 | 0.50 (0.30, 0.84) | 0.01 | 0.45 (0.25, 0.80) | 0.01 |
| Q3 (20.6 to <26.4) | 53/62 | 0.51 (0.28, 0.93) | 0.03 | 0.53 (0.27, 1.06) | 0.07 |
| Q4 (≥26.4) | 59/57 | 0.60 (0.32, 1.11) | 0.10 | 0.55 (0.27, 1.10) | 0.09 |
| Categories | | | | | |
| Q1 (<15.1) | 70/46 | *Ref* | | *Ref* | |
| Q2–Q4 (≥15.1) | 161/185 | 0.52 (0.32, 0.84) | 0.01 | 0.48 (0.28, 0.83) | 0.01 |
| Categories | | | | | |
| Q2–Q4 (≥15.1) | 161/185 | *Ref* | | *Ref* | |
| Q1 (<15.1) | 70/46 | 1.92 (1.20, 3.09) | 0.01 | 2.08 (1.20, 3.59) | 0.01 |

[a] Model 1 is conditioned on the matching factors of age, sex, treatment group, and study site. Model 2 is conditioned on the matching factors of age, sex, treatment group, and study site, and adjusted for BMI, smoking status, alcohol consumption, baseline SBP and DBP, time-averaged SBP and DBP during treatment, baseline fasting blood glucose, total cholesterol, triglycerides, season of blood collection, plasma calcium levels, folate, vitamin B12, vitamin A, vitamin E and vitamin K.

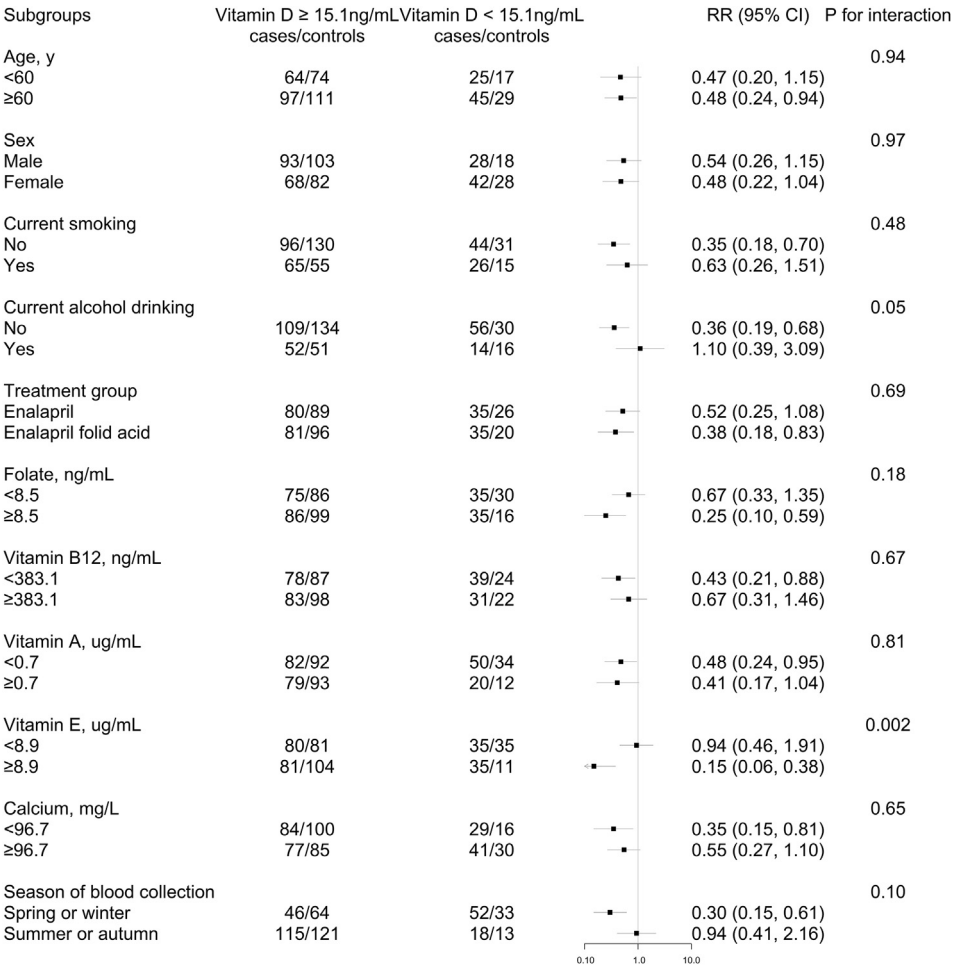


**Fig. 3.** The association between plasma 25-Hydroxyvitamain D concentrations (Q2–Q4 *vs.* Q1) and incident risk of total cancer in various subgroups. In addition to the matching factors (age, sex, treatment group, center), the models adjusted for BMI, smoking status, alcohol consumption, baseline SBP and DBP, time-averaged SBP and DBP during treatment, baseline fasting blood glucose, total cholesterol, triglycerides, calcium concentrations, season of blood collection, folate, vitamin B12, vitamin A, vitamin E and vitamin K, except for the stratifying variable.

remained robust even after adjustment for other vitamin status. Furthermore, our study extends the results of previously published related studies by demonstrating that the association between vitamin D and cancer risk can be significantly modified by vitamin E and alcohol intake.

Research findings to date on the association between plasma 25(OH) D and risk of total cancer remain inconsistent. The Health Professionals Follow-Up Study [23] showed that low concentrations of vitamin D may be associated with increased cancer incidence in men, particularly for digestive-system cancers. The

ESTHER study [24] also revealed an inverse association of 25(OH)D concentrations and total cancer risk in men but not in women. The Copenhagen City Heart Study [25] demonstrated that lower plasma 25(OH)D was associated with a higher risk of tobacco-related cancers, but not with risk of other cancers. However, some studies [14,15] found a U-shaped association between plasma 25(OH)D and cancer risk. Nevertheless, one cannot completely exclude the possibility that these findings could be due to chance or explained by uncontrolled or unknown confounding factors [26]. These discrepant findings might also be caused by differences in geographic locations, cancer types and population characteristics. These results underscore that the role of vitamin D in the development of cancer and its relation to known risk factors needs to be better understood and carefully considered in future study designs and data analyses.

Our study is the first of its kind conducted in Chinese hypertensive patients and has provided two new insights. First, our data suggest that in this population, the low vitamin D cut-off point marking an increased cancer risk was about 15 ng/mL, which is similar to that previously reported (14.3 ng/mL [27], 16.8 ng/mL [28], and 17.2 ng/mL [29]). This cut-off however, is substantially lower than what is currently recommended for bone health [30]. A target 25(OH)D concentration of 20 ng/mL was recommended by the bone-centric guidelines, while 30 ng/mL was recommended by the guidelines focused on pleiotropic effects [31]. Our findings echo the views that different cut-off values should be explored independently according to different health outcomes (i.e., cancer, cardiovascular disease, bone health, and metabolism) [30–32]. Furthermore, our study contributes a partial explanation for the null effect found in some previous randomized controlled trials, for those trials typically included participants with 25(OH) D concentrations greater than 15 ng/mL [16].

Second, vitamin E and alcohol drinking seemed to modify the association between plasma 25(OH)D and cancer risk. Vitamin E is a fat-soluble antioxidant and is postulated to reduce the risk of cancer [33]. It is possible that different vitamins may play various roles in the prevention of cancer and worth to explore both as individual and in combinations in relation to the risk of cancer. Alcohol consumption is a risk factor for cancer [34] and may also disturb vitamin D metabolism by decreasing the bioconversion of 25-hydroxyvitamin $D_3$ to calcitriol [35]. Thus, in our study, the stronger association between vitamin D and cancer risk was mainly observed in non-alcohol drinkers. Consistent with previous studies [36], we found more pronounced inverse association between vitamin D and cancer risk in participants whose blood samples were collected in spring or winter, but test for season * vitamin D interaction was not significant. Overall, our results were just hypothesis generating, and still need to be further investigated and confirmed in future studies.

While the exact mechanisms by which vitamin D is protective against cancer remains to be delineated, it is biologically plausible. The antineoplastic effects of vitamin D may stem from its active metabolite $1,25(OH)_2D$ (also known as calcitriol). Calcitriol binds to the vitamin D receptor (VDR), thereby creating a heterodimer with the retinoid X receptor (RXR), and associating with specific vitamin D–response elements (VDREs) to activate or inhibit target gene expression [37]. Various potential mechanisms of the antineoplastic actions of calcitriol on various malignancies have been demonstrated both in vitro and in vivo in animal models, including anti-proliferative effects, induction of apoptosis, stimulation of differentiation, anti-inflammatory effects, inhibition of invasion and metastasis, and inhibition of angiogenesis [38,39].

In our study, the mean plasma 25(OH)D concentration for all participants was 21.5 ng/mL. The prevalence of plasma 25 (OH) D <15, <20 and <30 ng/mL was 23.6%, 47.4% and 85.5%, respectively. In fact, vitamin D deficiency has been globally recognized as a public health problem [1,2]. Thus, our findings, if confirmed by future studies, may have important clinical and public health implications for Chinese populations and beyond.

Our study also had some other limitations. In our large and population-based cohort of hypertensive patients, the crude cancer incidence was about 249 per 100,000 patient-years, which is relatively lower than the age-specific incidence rates among older adults in the 2010 population-based cancer registration data, evaluated and analyzed by the National Central Cancer Registry (NCCR) of China [40]. This discrepancy may be related to differences in population characteristics, including blood pressure, gender distribution, urban-rural residences, smoking status, diet and lifestyle, etc. Our study was conducted in a rural population that was 59% female. The cancer incidence rates in rural areas and in females are lower than that in urban areas and in males [40]. Moreover, the prevalence of current smoking for males in our cohort was 52.6%, lower than that in the general population (for rural male residents, the prevalence of current smoking is 56.1%) [41]. What's more, diet and lifestyle may affect the risk of cancer. Therefore, there is an obvious geographical difference in cancer incidence in China. The top ten common cancers also differed by population and area [40]. In the CSPPT cohort, esophageal and gastric cancer were the first and second leading causes of cancer. Consistently, regional cancer registry data reported that stomach and esophageal cancer were the most frequently diagnosed cancers in rural Anhui and Jiangshu provinces in 2010 and 2013 [42,43]. In short, our current study was not a nationally representative sample. Whether our findings can be extrapolated to other areas with differences in diet and lifestyle still needs to be further investigated.

Recent studies on other populations showed that higher 25(OH) D concentrations were associated with lower breast cancer risk with concentrations beyond 60 ng/mL being most protective [19,44]. However, the concentrations of vitamin D in our population were generally low. As such, we are unable to examine the 25(OH) D-cancer association among those with high 25(OH)D concentrations.

Our study was limited by relatively small sample sizes for cases and controls, which was underpowered to investigate the impact of plasma 25(OH)D concentrations on risk of different subtypes of cancer. Furthermore, the current study was a nested case–control study with a 1:1 ratio for cases and controls. In future studies, a nested case–control study with a $1:n$ ($>1$) ratio may have improved the statistical power. We would like to emphasize that our study is just hypotheses generating. Our findings warrant additional investigations, including large-scale cohort studies and randomized trials.

Circulating 25(OH)D concentrations were only assessed at baseline. More frequent measurements would possibly have provided more information. Finally, our study included hypertensive adults living in a central area of China (ranging from 30.1°N to 34.8°N latitude). The annual cloud-adjusted ambient solar UVB irradiance at 305 nm ranged from 43 to 51 in this area [10]. Whether the observed results can be extrapolated to other populations with different sun exposures will require further verification.

In conclusion, in this nested case–control study, we found that low plasma 25(OH) D concentrations (<15 ng/mL) were associated with an increased risk of incident cancer among Chinese hypertensive adults; conversely, higher plasma 25(OH)D concentrations (≥15.1 ng/mL, quartiles 2–4) were associated with a lower risk of incident cancer in comparison to those with lower concentrations. The associations were robust after adjusting for potential covariables and were consistent across subtypes of cancer. Several potential effect modifiers were identified, including plasma vitamin E

concentrations and alcohol intake. These findings warrant additional investigation.

## Conflict of interest

Dr. Yong Huo reports grants from the National Key Research and Development Program (2016YFC0903103). Dr. Xiping Xu reports grants from the National Key Research and Development Program (2016YFE0205400, 2018ZX09739, 2018ZX09301034003), the Science and Technology Planning Project of Guangzhou, China (201707020010), and the Science, Technology and Innovation Committee of Shenzhen (JSGG20170412155639040, GJHS20170314114526143). Dr. Xianhui Qin reports grants from the Presidential Foundation of Nanfang Hospital, Southern Medical University (2017C007), and the Outstanding Youths Development Scheme of Nanfang Hospital, Southern Medical University (2017J009). Dr. Huiyuan Guo reports grants from the 111th Project from the Education Ministry of China (No. B18053).

## CRediT authorship contribution statement

**Tengfei Lin:** Formal analysis, Investigation, Visualization, Writing - original draft. **Yun Song:** Investigation, Writing - original draft. **Xianglin Zhang:** Investigation. **Huiyuan Guo:** Investigation. **Lishun Liu:** Data curation, Investigation. **Ziyi Zhou:** Investigation. **Binyan Wang:** Investigation. **Genfu Tang:** Investigation. **Chengzhang Liu:** Investigation, Validation. **Yan Yang:** Writing - review & editing. **Wenhua Ling:** Writing - review & editing. **Zhengqiang Yuan:** Writing - review & editing. **Jianping Li:** Investigation, Resources. **Yan Zhang:** Investigation, Resources. **Yong Huo:** Conceptualization, Resources, Project administration. **Xiaobin Wang:** Conceptualization, Writing - original draft, Writing - review & editing. **Hao Zhang:** Conceptualization, Writing - original draft, Writing - review & editing. **Xianhui Qin:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Xiping Xu:** Conceptualization, Methodology, Writing - review & editing.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clnu.2018.10.019.

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