RESEARCH ARTICLE

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# *Trans*-Lycopene and *β*-Cryptoxanthin Intake and Stomach Cancer in Vietnamese Men: A Pilot Case-Control Study

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## Abstract

**Objective:** To examine the association between dietary intake of *Trans*-Lycopene and *β*-Cryptoxanthin and stomach cancer in Vietnamese men. **Methods:** A case-control study including 80 male incident stomach cancer cases and 146 male controls was performed in a general hospital in Viet Nam. A validated semi-quantitative food frequency (SQFFQ) and demographic lifestyle questionnaire were designed, and venous blood samples were collected to determine *H. pylori* status by IgG ELISA. Nutrient intake was converted using the data of SQFFQ and the Nutritive Composition Table of Vietnamese Foods, updated in 2019. The respective associations between *Trans*-Lycopene and *β*-Cryptoxanthin intake and stomach cancer were examined using unconditional logistic regression analysis with adjustments for possible cofactors. **Results:** Both *Trans*-Lycopene and *β*-Cryptoxanthin intake and stomach cancer showed a significantly inverse association, tertile-3 versus tertile-1, (OR = 0.15, 95%CI: 0.06–0.35, p trend = 0.00) and (OR = 0.34, 95%CI: 0.14–0.79, p trend = 0.02, respectively). For *Trans*-Lycopene intake stratifying by *H. pylori* status remained the benefit effect against stomach cancer among *H. pylori*-negative participants (OR = 0.15, 95%CI: 0.03–0.69, p trend = 0.02) and *H. pylori*-positive participants (OR = 0.13, 95%CI: 0.04–0.42, p trend = 0.00). **Conclusions:** Both *Trans*-Lycopene and *β*-Cryptoxanthin intake showed a strong protective effect against stomach cancer. The findings suggest that these two types of fat-soluble micronutrients would be considered as an anti-cancer therapy for both primary and secondary prevention.

**Keywords:** Stomach cancer- *Helicobacter pylori*- *Trans*-Lycopene and *β*-Cryptoxanthin intake

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## Introduction

*Trans*-Lycopene and *β*-Cryptoxanthin are fat-soluble micronutrients and carotenoids and are found in many foods, including some vegetables, fruits, meats, and animal products. At least 700 carotenoids are found in natural foods (Cited by CDC) (CDC, 2008). Carotenoids are believed among the best biological markers for fruits and vegetable intake. The strongest dietary predictors of serum carotenoid concentration are fruits for sources of *β*-Cryptoxanthin, and tomato products for *Trans*-Lycopene (CDC, 2008; CDC, 2012). Both vegetables and fruits are protective against many cancer sites(WCRF, 2007). We hypothesized that *Trans*-Lycopene and *β*-Cryptoxanthin intake may play an important role to reduce the risk of developing stomach cancer in humans. The present study aimed to examine the association between dietary intake of *Trans*-Lycopene and *β*-Cryptoxanthin and stomach cancer in Vietnamese men.

## Materials and Methods

*Methods*

A case-control study including 80 male incident stomach-cancer cases and 146 male controls was performed in Bach Mai general university hospital located in the city of Hanoi in the North Viet Nam during 2018-2019, Figure 1.

*Cases and hospital controls recruitments*

The incident stomach-cancer cases were first diagnosed as having stomach cancer by histopathological confirmation and underwent surgery to remove the cancer tumor. The male hospital controls were matching to the cases by +/-5-year-old and admitted into the same surgery department and also undergone surgery on to treat for non-cancer tumor. The patients were able to well communicate to answer study questions by face-to-face interviews by the trained data collectors on times before the operating day. Both cases and hospital controls were agreed upon and signed informed consent to participate in the present study.

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The control-per-case ratio was preferable to about two.

*Exposure assessments*

A validated semi-quantitative food frequency (SQFFQ) and demographic lifestyle questionnaire were designed including 113 questions. Additional 79 questions were added to collect data on cooking methods and pre-clinical examination results. Nutrient intake was converted using the data of SQFFQ and the Nutritive Composition Table of Vietnamese Foods, updated in 2019. The SQFFQ inquired about dietary habits during the previous one year for 85 foods/recipes and frequency in 7 categories: seldom or never intake, 6-11 times/year, 1-3 times/month, 1-2 times/week, 3-4 times/week, 5-6 times/week, and 1-3 times/day. Nutrients intake was estimated following the formulation, based on the Nutritive Composition Table of Vietnamese Foods, updated in 2019, such as:

$$n=f*p*(w/100)*k/365$$

Where "f" was frequency intake during the last year; "p" was the amount of nutrient per 100 gram; "w" was average weight intake of one time or service; "k" was the weighted size of intake amount (small=0.8, medium=1.0, large=1.2). The designed SQFFQ was being validated as having a good characteristic of feasibility, practicality, and reliability in the general populations located in North Viet Nam (Le et al., 2018).

For tobacco smoking, participants were asked about the types of tobacco (cigarette, waterpipe tobacco, others); the average number of tobacco consumed per day during their age of 15-20, 21-15, 26-30, 31-40, 41-50, 51-60, 61-70, and 71+ (if applicable); the duration of smoking (for the current smoker); and the duration of quit smoking (for current and ex-smokers).

To investigate the presence of *H. pylori* infection, 3 ml aliquots of overnight fasting venous blood were collected from both cases and controls. The blood tube was shaken gently several times to mix blood and EDTA-2Na, quickly followed by centrifugation at 3,000 rpm for 20 minutes in a centrifuge with low-temperature control. The anti-*H. pylori* serum IgG titers were tested by enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle using *H. pylori* IgG ELISA kit (RE56381) from IBL International (Hamburg, Germany). The experienced investigators of the Microbiology and Infectious Disease, College of Veterinary Medicine, Vietnam National University of Agriculture performed laboratory work for all plasma samples with the ID of study participants only to avoid information bias and systematic errors in determining the status of *H. pylori* infection.

Data analysis

The respective associations between *Trans*-Lycopene and *β*-Cryptoxanthin intake and stomach cancer were examined using unconditional logistic regression analysis with adjustments for possible cofactors of the age groups (20-49, 50-59, 60-69, ≥70 ages), education levels (primary school, secondary school, high school or higher), BMI (<18.5, 18.5 to <23, 23 to <25, ≥25 kg/m$^2$), tobacco smoking (ever/never smokers), consumption of five common types of vegetables (water spinach, mustard greens, sauropods, Malabar nightshade, cabbage); and *H. pylori* infection status, total calorie intake, and family history of cancer. Sensitive analysis was done for the sub-groups of tobacco smoking (ever/never smokers) and *H. pylori* infection status. All p-values were two-sided, and $p \leq 0.05$ (alpha value) was considered to indicate statistical significance.

## Results

*H. pylori*-positive was 50.00% of cases and 38.36% of controls. Ever tobacco smokers were 88.75% of cases and 73.97% of controls. Normal body-mass-index (18.5-<23.0) was 52.5% of cases and 50.00% of controls. Less than 6-year education was 18.75% of cases and 12.33% of controls, Table 1.

Both *Trans*-Lycopene and *β*-Cryptoxanthin intake and stomach cancer showed a significantly inverse association, tertile-3 versus tertile-1, (OR = 0.15, 95%CI: 0.06–0.35, p

Table 1. Characteristics of the Study Population

| Variables | Stomach cancer | | Non-cancer | |
|---|---|---|---|---|
| | n | % | n | % |
| Age group (ages) | | | | |
| 20-49 | 14 | 17.50 | 47 | 32.19 |
| 50-59 | 24 | 30.00 | 53 | 36.3 |
| 60-69 | 28 | 35.00 | 31 | 21.23 |
| ≥70 | 14 | 17.50 | 15 | 10.27 |
| Total | 80 | 100.00 | 146 | 100.00 |
| Education (years) | | | | |
| <6 | 15 | 18.75 | 18 | 12.33 |
| 6-9 | 36 | 45.00 | 64 | 43.84 |
| 10-12 | 15 | 18.75 | 46 | 31.51 |
| >12 | 13 | 16.25 | 17 | 11.64 |
| Unknown | 1 | 1.25 | 1 | 0.68 |
| Total | 80 | 100.00 | 146 | 100.00 |
| BMI (kg/m$^2$)[a] | | | | |
| 18.5 to <23 | 42 | 52.5 | 73 | 50.00 |
| 23 to <25 | 9 | 11.25 | 30 | 20.55 |
| ≥25 | 5 | 6.25 | 18 | 12.33 |
| <18.5 | 24 | 30 | 22 | 15.07 |
| Unknown | 0 | 0.00 | 3 | 2.05 |
| Total | 80 | 100.00 | 146 | 100.00 |
| Lifetime tobacco smoking | | | | |
| Never | 9 | 11.25 | 38 | 26.03 |
| Ever | 71 | 88.75 | 108 | 73.97 |
| Total | 80 | 100.00 | 146 | 100.00 |
| *H. pylori* serostatus [b] | | | | |
| Negative (COI <0.8) | 25 | 31.25 | 63 | 43.15 |
| Equivocal (COI 0.8-1.2) | 15 | 18.75 | 27 | 18.49 |
| Positive (COI >1.2) | 40 | 50.00 | 56 | 38.36 |
| Total | 80 | 100.00 | 146 | 100.00 |

[a], BMI, Body Mass Index (BMI= weight (kg) / height ((m)$^2$); [b], Classification according to the manufacturer's instructions according to anti-*H. pylori* IgG concentration of the Cut-off Index (COI) quantitative

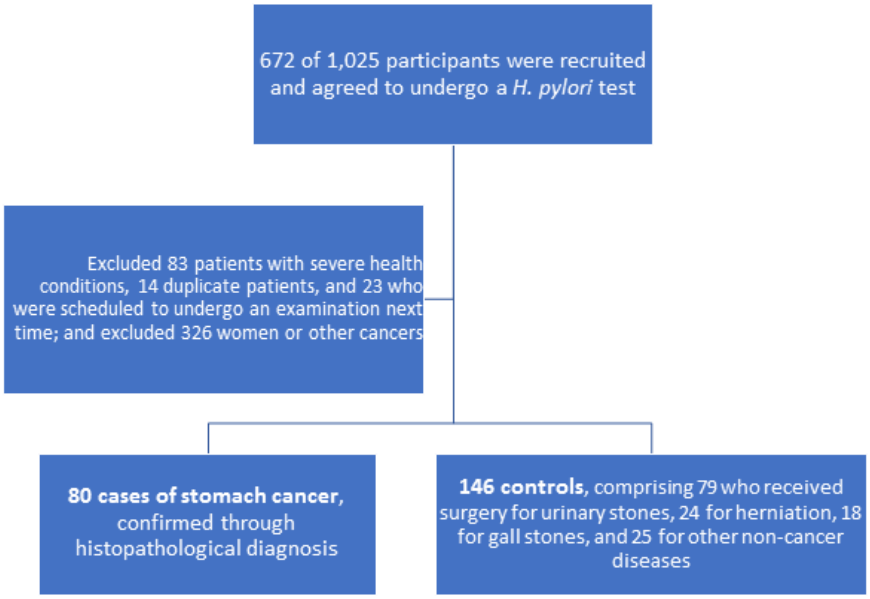


Figure 1. Flow Chart of Recruited Study Participants during 2018-2019.

Table 2. Daily Intake of Trans-Lycopene and Stomach Cancer in Vietnamese Men

| Total | Daily mean intake (µg) | Control | Case | Total | OR (95%CI) | P for trend |
|---|---|---|---|---|---|---|
| Total & | | | | | | |
| | 332.6 | 38 | 38 | 76 | 1 | |
| | 752.8 | 47 | 30 | 77 | 0.53 (0.26, 1.08) | |
| | 1538.3 | 61 | 12 | 73 | 0.15 (0.06, 0.35) | |
| | Total | 146 | 80 | 226 | | 0 |
| Never smoker && | | | | | | |
| | 334 | 9 | 5 | 14 | 1 | |
| | 793.7 | 10 | 2 | 12 | 0.17 (0.01, 1.97) | |
| | 1671.2 | 19 | 2 | 21 | 0.21 (0.02, 2.46) | |
| | Total | 38 | 9 | 47 | | 0.18 |
| Ever smoker && | | | | | | |
| | 332.3 | 29 | 33 | 62 | 1 | |
| | 745.2 | 37 | 28 | 65 | 0.57 (0.26, 1.23) | |
| | 1484.7 | 42 | 10 | 52 | 0.14 (0.05, 0.37) | |
| | Total | 108 | 71 | 179 | | 0 |
| *H. pylori* negative $ | | | | | | |
| | 372.5 | 17 | 12 | 29 | 1 | |
| | 710.9 | 16 | 8 | 24 | 1.08 (0.28, 4.27) | |
| | 1599.5 | 30 | 5 | 35 | 0.15 (0.03, 0.69) | |
| | Total | 63 | 25 | 88 | | 0.02 |
| *H. pylori* positive or equivocal $ | | | | | | |
| | 308 | 21 | 26 | 47 | 1 | |
| | 771.8 | 31 | 22 | 53 | 0.37 (0.15, 0.91) | |
| | 1482 | 31 | 7 | 38 | 0.13 (0.04, 0.42) | |
| | Total | 83 | 55 | 138 | | 0 |

OR (95% CI): odds ratio 95% confidence interval. &: Adjusted for age groups (20-49, 50-59, 60-69, ≥70 ages), education levels (primary school, secondary school, high school or higher), BMI (<18.5, 18.5 to <23, 23 to <25, ≥25 kg/m2), tobacco smoking (ever/never smokers), consumption of five common types of vegetables (water spinach, mustard greens, sauropods, Malabar nightshade, cabbage); and H. pylori infection status, total calorie intake, and family history of cancer. &&: Adjusted for age groups (20-49, 50-59, 60-69, ≥70 ages), education levels (primary school, secondary school, high school or higher), BMI (<18.5, 18.5 to <23, 23 to <25, ≥25 kg/m2), consumption of five common types of vegetables (water spinach, mustard greens, sauropods, Malabar nightshade, cabbage); and H. pylori infection status, total calorie intake, and family history of cancer. $: Adjusted for age groups (20-49, 50-59, 60-69, ≥70 ages), education levels (primary school, secondary school, high school or higher), BMI (<18.5, 18.5 to <23, 23 to <25, ≥25 kg/m2), tobacco smoking (ever/never smokers), consumption of five common types of vegetables (water spinach, mustard greens, sauropods, Malabar nightshade, cabbage), total calorie intake, and family history of cancer;

Table 3. Daily Intake of *β*-Cryptoxanthin and Stomach Cancer in Vietnamese Men

| Total | Daily mean intake (μg) | Control | Case | Total | OR (95%CI) | P for trend |
|---|---|---|---|---|---|---|
| Total & | | | | | | |
| | 24.1 | 46 | 30 | 76 | 1 | |
| | 81.4 | 52 | 37 | 89 | 0.73 (0.36, 1.47) | |
| | 148.2 | 48 | 13 | 61 | 0.34 (0.14, 0.79) | |
| | Total | 146 | 80 | 226 | | 0.02 |
| Never smoker && | | | | | | |
| | 27.9 | 14 | 4 | 18 | 1 | |
| | 73.5 | 9 | 4 | 13 | 0.82 (0.09, 7.29) | |
| | 157.8 | 15 | 1 | 16 | 0.23 (0.02, 2.87) | |
| | Total | 38 | 9 | 47 | | 0.26 |
| Ever smoker && | | | | | | |
| | 23 | 32 | 26 | 58 | 1 | |
| | 82.8 | 43 | 33 | 76 | 0.66 (0.31, 1.42) | |
| | 144.7 | 33 | 12 | 45 | 0.36 (0.14, 0.91) | |
| | Total | 108 | 71 | 179 | | 0.03 |
| *H. pylori*-negative $ | | | | | | |
| | 25.6 | 18 | 8 | 26 | 1 | |
| | 79.2 | 20 | 10 | 30 | 1.03 (0.28, 3.76) | |
| | 158.2 | 25 | 7 | 32 | 0.51 (0.13, 2.02) | |
| | Total | 63 | 25 | 88 | | 0.35 |
| *H. pylori*-positive or equivocal $ | | | | | | |
| | 23.3 | 28 | 22 | 50 | 1 | |
| | 82.5 | 32 | 27 | 59 | 0.56 (0.23, 1.38) | |
| | 137.1 | 23 | 6 | 29 | 0.19 (0.06, 0.65) | |
| | Total | 83 | 55 | 138 | | 0.01 |

OR (95% CI): odds ratio 95% confidence interval. &: Adjusted for age groups (20-49, 50-59, 60-69, ≥70 ages), education levels (primary school, secondary school, high school or higher), BMI (<18.5, 18.5 to <23, 23 to <25, ≥25 kg/m2), tobacco smoking (ever/never smokers), consumption of five common types of vegetables (water spinach, mustard greens, sauropods, Malabar nightshade, cabbage); and H. pylori infection status, total calorie intake, and family history of cancer. &&: Adjusted for age groups (20-49, 50-59, 60-69, ≥70 ages), education levels (primary school, secondary school, high school or higher), BMI (<18.5, 18.5 to <23, 23 to <25, ≥25 kg/m2), consumption of five common types of vegetables (water spinach, mustard greens, sauropods, Malabar nightshade, cabbage); and H. pylori infection status, total calorie intake, and family history of cancer. $: Adjusted for age groups (20-49, 50-59, 60-69, ≥70 ages), education levels (primary school, secondary school, high school or higher), BMI (<18.5, 18.5 to <23, 23 to <25, ≥25 kg/m2), tobacco smoking (ever/never smokers), consumption of five common types of vegetables (water spinach, mustard greens, sauropods, Malabar nightshade, cabbage), total calorie intake, and family history of cancer;

trend = 0.00) and (OR = 0.34, 95%CI: 0.14–0.79, p trend = 0.02, respectively). For *Trans*-Lycopene intake stratifying by *H. pylori* status remained the benefit effect against stomach cancer among *H. pylori*-negative participants (OR = 0.15, 95%CI: 0.03–0.69, p trend = 0.02) and *H. pylori*-positive participants (OR = 0.13, 95%CI: 0.04–0.42, p trend = 0.00). By smoking habits, a significant negative association between *Trans*-Lycopene intake and stomach cancer was observed among ever smokers (tertile-3 versus tertile-1, OR = 0.14, 95%CI: 0.05–0.37, p for trend = 0.00), but no such statistically significant relationship was found among never smokers (OR = 0.21, 95%CI: 0.02–2.46, p for trend = 0.18), Table 2.

For β-Cryptoxanthin intake by *H. pylori* status, the benefit effect against stomach cancer was not observed among *H. pylori*-negative participants (OR = 0.51, 95%CI: 0.13–2.02, p trend = 0.35) but that was seen among *H. pylori*-positive participants (OR = 0.19, 95%CI: 0.06–0.65, p trend = 0.01). By smoking habits, a significant negative association between β-Cryptoxanthin intake and stomach cancer was observed among ever smokers (OR = 0.36, 95%CI: 0.14–0.91, p for trend = 0.03), but no such statistically significant relationship was found among never smokers (OR = 0.23, 95%CI: 0.02–2.87, p for trend = 0.26), Table 3.

## Discussion

We observed a strong protective effect against stomach cancer by both two natural micronutrients of *Trans*-Lycopene and *β*-Cryptoxanthin intake and this activity strongly remained among *H. pylori*-positive participants and ever-smoker individuals. The findings suggest that these two types of fat-soluble micronutrients would be considered as an anti-cancer therapy for primary prevention in controlling the occurrence and mortality from stomach cancer.

The present findings are supporting data of *Trans*-Lycopene and *β*-Cryptoxanthin regarding their roles of antioxidant activity, anti-inflammatory activity, and

anti-cancer. The carotenoids are fat-soluble micronutrients that are found in many foods of vegetables, fruits, meats, and animal products. Six major carotenoids are found in human blood including alpha-carotene, beta-carotene, Beta-cryptoxanthin, lutein, *Trans*-lycopene, and zeaxanthin (Li et al., 2021). Carotenoids are believed among the best biological markers for fruit and vegetable intake. *Trans*-Lycopene, molecular formula C40H56, is a natural strong antioxidant to protect against cancer. Major *Trans*-Lycopene is obtained primarily from tomato and other pink orange or red fruits and vegetables mainly including guava, papaya, peaches, pink grapefruit, red carrots, gac melons, watermelons, apricot, cranberry. Academic reports have found that lycopene has antioxidant, anti-inflammatory, anti-carcinogenic (Li et al., 2021). However, the association between dietary lycopene intake and stomach cancer is inconclusive (Yang et al., 2013), (Ito et al., 2005). Beta-cryptoxanthin as pro-vitamin A is an essential nutrient supporting the growth development and maintenance of the immune system. Beta-cryptoxanthin can do antioxidant activity, anti-inflammatory activity, and anti-cancer (Burri et al., 2016).

Stomach cancer (or gastric cancer) is the fifth most frequent cancer incidence and the third leading cancer death globally (GLOBOCAN 2018) (Bray et al., 2018). In Asia, stomach cancer is the third most common cancer and the second most common cause of cancer death with the highest incidence rates were observed in the Eastern and Southeastern regions (Colquhoun et al., 2012). The present findings add data to promote cancer prevention based on a healthy diet and intake of natural food rich in *Trans*-Lycopene and *β*-Cryptoxanthin.

The present study has certain limitations of the absent data in women and limited sample size as a pilot case-control study in the low- and middle economy country of Viet Nam. The other possible limitation is that there were available hospital controls only and a recall of dietary habits might be a low accuracy and completeness of food frequency intake. Despite these limitations, the results support the hypothesis of *Trans*-Lycopene and β-Cryptoxanthin intake may play an important role to reduce the risk of developing stomach cancer in humans.

## Author Contribution Statement

Conceptualization: KS, THH, DVH, LTN. Data curation: NTL. Formal analysis: NTL. Funding acquisition: NTL. Methodology: NTL, KS. Project administration: DVH, NTL. Visualization: THH, NTL. Writing - original draft: NTL. Writing - review & editing: DVH, NTL

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*Available data*

The original database will be available upon appropriate request.

*Conflict of interest*

There are no conflicts to disclose

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