

## Glucosinolate and isothiocyanate intakes are inversely associated with breast cancer risk: a case–control study in China

Nai-Qi Zhang<sup>1,2</sup>, Suzanne C. Ho<sup>3</sup>, Xiong-Fei Mo<sup>4</sup>, Fang-Yu Lin<sup>5</sup>, Wu-Qing Huang<sup>1</sup>, Hong Luo<sup>1</sup>, Jing Huang<sup>1</sup> and Cai-Xia Zhang<sup>1,2\*</sup>

<sup>1</sup>Department of Medical Statistics and Epidemiology, School of Public Health, Sun Yat-sen University, Guangzhou 510080, People's Republic of China

<sup>2</sup>Guangdong Provincial Key Laboratory of Food, Nutrition and Health, School of Public Health, Sun Yat-sen University, Guangzhou 510080, People's Republic of China

<sup>3</sup>Division of Epidemiology, The Jockey Club School of Public Health and Primary Care, The Chinese University of Hong Kong, Hong Kong SAR, People's Republic of China

<sup>4</sup>Department of Thyroid and Breast Surgery, the First Affiliated Hospital of Sun Yat-sen University, Guangzhou 510080, People's Republic of China

<sup>5</sup>Nursing Department, the First Affiliated Hospital of Sun Yat-sen University, Guangzhou 510080, People's Republic of China

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### Abstract

Although previous studies have investigated the association of cruciferous vegetable consumption with breast cancer risk, few studies focused on the association between bioactive components in cruciferous vegetables, glucosinolates (GSL) and isothiocyanates (ITC), and breast cancer risk. This study aimed to examine the association between consumption of cruciferous vegetables and breast cancer risk according to GSL and ITC contents in a Chinese population. A total of 1485 cases and 1506 controls were recruited into this case–control study from June 2007 to March 2017. Consumption of cruciferous vegetables was assessed using a validated FFQ. Dietary GSL and ITC were computed by using two food composition databases linking GSL and ITC contents in cruciferous vegetables with responses to the FFQ. The OR and 95% CI were assessed by unconditional logistic regression after adjusting for the potential confounders. Significant inverse associations were found between consumption of cruciferous vegetables, GSL and ITC and breast cancer risk. The adjusted OR comparing the highest with the lowest quartile were 0.51 (95% CI 0.41, 0.63) for cruciferous vegetables, 0.54 (95% CI 0.44, 0.67) for GSL and 0.62 (95% CI 0.50, 0.76) for ITC, respectively. These inverse associations were also observed in both premenopausal and postmenopausal women. Subgroup analysis by hormone receptor status found inverse associations between cruciferous vegetables, GSL and ITC and both hormone-receptor-positive or hormone-receptor-negative breast cancer. This study indicated that consumption of cruciferous vegetables, GSL and ITC was inversely associated with breast cancer risk among Chinese women.

**Key words:** Cruciferous vegetables: Glucosinolates: Isothiocyanates: Breast cancer: Case–control studies

Breast cancer is the most common cancer in women, and ranks as the leading cause of cancer-related death among women worldwide. In all, 1.7 million breast cancer cases consisted of one-quarter of the emerging malignant tumours, and 521 900 deaths occurred in 2012<sup>(1)</sup>. Although the incidence of breast cancer has been growing worldwide in the past years, the incidence in Asian countries is still lower compared with that in Western countries<sup>(2)</sup>. Studies have shown evidence that the increasing breast cancer risk is associated with dietary factors, which could be a safe and cost-effective method for cancer prevention.

Cruciferous vegetables have been of great interest. Numerous meta-analyses have shown the beneficial effect of cruciferous

vegetables on breast, lung, colorectal, gastric, pancreatic, renal, prostate and other cancers<sup>(3–11)</sup>. The anti-carcinogenic effect of cruciferous vegetables against various cancers may be owing to the high levels of glucosinolates (GSL)<sup>(12)</sup>, which can hydrolyse into indole-3-carbinol and isothiocyanates (ITC) by plant myrosinase and gastrointestinal microflora. Although GSL have no direct biological activity, its major hydrolysis products exert their anti-carcinogenic effects *in vivo* by multiple mechanisms, including inhibiting the activity of carcinogen-activating enzymes, expediting apoptosis and arresting cell cycle progression<sup>(13)</sup>. ITC, in particular, is able to suppress the activation of carcinogen by cytochromes *P450* (CYP) by down-regulating

**Abbreviations:** ER, oestrogen receptor; ER+, ER positive; ER–, ER negative; GSL, glucosinolate; ITC, isothiocyanate; PR, progesterone receptor; PR+, PR positive; PR–, PR negative.

\* **Corresponding author:** Professor C.-X. Zhang, fax +86 20 87330446, email zhangcx3@mail.sysu.edu.cn

the enzyme levels and directly inhibiting their catalytic activities. In addition, ITC can induce glutathione *S*-transferase (GST), one of the most crucial phase II enzymes, which can effectively detoxify electrophilic compounds generated by phase I enzymes and further destroy their ability to damage DNA<sup>(14)</sup>. As the main sources of these compounds are easily available in traditional Chinese diets, these agents are ideal for potential effective chemoprotection of humans against cancer.

One meta-analysis published in 2013 suggested that high consumption of cruciferous vegetables may reduce the risk of breast cancer<sup>(10)</sup>. Only one cohort study found the inverse association between consumption of GSL and prostate cancer<sup>(15)</sup>. Two case-control studies observed that higher ITC intake was associated with lower lung cancer risk<sup>(16–17)</sup>. However, no epidemiological study has yet carried out systematic investigation between total cruciferous vegetables, estimated GSL and ITC content consumption and the risk of breast cancer. Thus, we performed a case-control study to evaluate the association between cruciferous vegetable intakes and breast cancer risk according to GSL and ITC content in a Chinese population. We hypothesised that high intake of cruciferous vegetables, GSL and ITC was inversely associated with breast cancer risk.

## Methods

### Study population

A two-stage hospital-based case-control study was conducted to recruit breast cancer cases and controls. Detailed study methods of the first-stage case-control study design have been reported elsewhere<sup>(18–19)</sup>. To be brief, the first stage was conducted from June 2007 to August 2008 and the second stage from September 2011 to March 2017. Eligible women aged 25–70 years, Guangdong natives or those who have lived in Guangdong province for at least 5 years with incident, primary, histologically confirmed breast cancer diagnosed no more than 3 months before the interview were recruited from three major hospitals in Guangzhou. Subjects were excluded if they could not understand or speak Mandarin or Cantonese or had a past history of cancers. Totally, 1614 eligible cases were identified, of which 1485 (92.0%) were successfully interviewed.

Controls were admitted to the same hospitals as cases during the same time period, frequency-matched by age (5-year interval) with cases. Control subjects shared the same eligibility criteria with cases, except that they had no past history of any cancer. They were selected from the Departments of Ophthalmology, Plastic and Reconstructive Surgery, Vascular Surgery, Ear-Nose-Throat, and Orthopedics and Microsurgery. In total, 1506 (92.4%) controls out of 1630 eligible controls participated in this study.

We assumed that people with a higher intake of cruciferous vegetables represented 25% of the general population, the estimated OR between the consumption of cruciferous vegetables and breast cancer risk was 0.49<sup>(19)</sup>, the type I error rate was <0.05 ( $\alpha = 0.05$ ), the power of test was 90% ( $\beta = 0.10$ ) and the response rate was 90%. On the basis of these assumptions, we require a sample size of 304 cases.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving human subjects were approved by The Ethical Committee of School of Public Health, Sun Yat-sen University. Written informed consents were obtained from all participants.

### Data collection

A face-to-face interview was performed by trained interviewers by using a structured questionnaire, which comprised dietary habits, socio-demographic factors, anthropometric factors, lifestyle behaviours, family history of cancer and reproductive information. In this study, regular smokers were defined as those smoking at least 1 cigarette/d for more than 6 consecutive months. Passive smokers were defined as non-smokers who reported being exposed to others' tobacco smoke at least 15 min/d in the previous 5 years. Regular drinkers were defined as those drinking alcohol at least once per week in the past 5 years. Women were identified as postmenopausal if they had cessation of menstrual period for at least 12 months since the last menstrual cycle. BMI was calculated by dividing weight (kg) by height squared (m<sup>2</sup>). Data on current occupational activity were obtained on the basis of self-reported employment status and the level of physical activity done at work (non-working, sedentary, standing, manual, heavy manual). A metabolic equivalent (MET) value was assigned to each reported activity based on the Compendium<sup>(20–21)</sup>. MET-h per week (how many days per week  $\times$  how many hours per day  $\times$  MET for a specific activity) were calculated for the frequency (d/week) and typical duration (h/d) of household activities (mopping, cooking and so on) and recreational activities (walking, jogging, climbing, running, playing table tennis and so on) during the past year. Relevant medical information, medical diagnosis, histological findings and oestrogen receptor (ER) and progesterone receptor (PR) status were extracted from the hospital medical records.

Cruciferous vegetable intakes were assessed using a validated ninety-one-item FFQ, which investigated the habitual diet of all the subjects during the previous year before diagnosis for cases or before the time of interview for controls. The validity and reliability of FFQ have been described previously<sup>(22)</sup>. Each participant was asked the frequency, on average, of each type of food they consumed over the previous year. Food photographs about different portion size of foods were used to help the participants to quantify the amount they consumed. Nutrient values intakes were estimated using the 2002 Chinese Food Composition Table<sup>(23)</sup>. A total of ten types of cruciferous vegetables common in Guangdong province were listed on the FFQ, including choi sum, kaichoi, kailan, broccoli, pakchoi, bokchoi, cabbage, mini Chinese cabbage, cauliflower and radish. On the basis of the reported intake information, the daily average intake of total cruciferous vegetable was calculated. Furthermore, dietary GSL was computed by linking food consumption data to a food composition database<sup>(24)</sup>, which summarised eighteen studies to form a database on total GSL contents in cruciferous vegetables. The estimated weekly intake of ITC was calculated by using previously published ITC concentrations for cruciferous vegetables grown in Singapore and cooked in boiling water<sup>(25)</sup> by linkage of ITC contents in cruciferous vegetables with responses to the FFQ.



**Statistical analysis**

Subjects were categorised into quartiles based on the distribution of cruciferous vegetables or nutrients among the controls. The lowest quartile served as the reference group in the analyses. Student's *t* tests were used for continuous variables (such as age, BMI, household and recreational activities, age at menarche) and  $\chi^2$  tests were used for categorical variables (such as income, educational level, smoking status) to test differences between different groups. Dietary cruciferous vegetables and nutrient intake were adjusted for total energy intake by using the residual method<sup>(26)</sup>. OR and 95 % CI summarising the association between cruciferous vegetables or nutrients and breast cancer risk were calculated by using unconditional logistic regression adjusting for age, education, income, BMI, regular smoking, passive smoking, regular drinking, household and recreational activities, occupational activity, age at menarche, first-degree relative with cancer and ever used an oral contraceptive. Confounding factors were selected by comparing baseline characteristics between the cases and controls. Tests for trend were performed by entering the categorical variables as continuous parameters in the models.

As menopause is the marker of cessation of ovarian function and related to endocrine activity, and late menopause is a known risk factor for breast cancer<sup>(27)</sup>, stratified analysis by menopausal status (premenopausal and postmenopausal) was conducted to assess the associations between cruciferous vegetable or nutrient intakes and breast cancer risk. Subgroup analysis by sex hormone receptor status (ER positive (ER+) or ER negative (ER-); PR positive (PR+) or PR negative (PR-)) was also conducted. All of the aforementioned statistical analyses were carried out using IBM SPSS Statistics, version 21.0.

**Results**

The socio-demographic and established breast cancer risk factors of the study subjects are shown in Table 1. Compared with controls, breast cancer cases were more likely to have less education and lower income. More cases reported having a first-degree relative with cancer, a history of using oral contraceptive, higher BMI and younger age at menarche. A higher proportion of cases tended to drink and smoke regularly and be exposed to second-hand smoke, engaged in fewer household and leisure activities and more occupational activities. These variables were considered potential confounders and adjusted for in the subsequent multivariable analyses. Age was also adjusted in the model as matching was on 5-year intervals. No significant differences were observed between cases and controls regarding age, marital status, occupation, age at first live birth, parity, menopausal status and breast-feeding history.

Among control subjects, mean intakes were 130.53 (SD 68.93) g/d for energy-adjusted total cruciferous vegetables, 100.94 (SD 50.80) mg/d for energy-adjusted GSL and 32.10 (SD 20.79)  $\mu$ mol/d for energy-adjusted ITC. Compared with controls, cases tended to have lower intakes of total cruciferous vegetables, GSL and ITC (Table 1).

A strong inverse association was found between total cruciferous vegetable intake and breast cancer risk (Table 2). The OR for the highest quartile of intake in comparison with the lowest

**Table 1.** Socio-demographic characteristics and selected risk factors of breast cancer in the study population (Mean values and standard deviations; numbers and percentages)

	Cases (n 1485)		Controls (n 1506)		P
	Mean	SD	Mean	SD	
Age (years)	47.56	9.47	47.58	9.45	0.955
BMI (kg/m <sup>2</sup> )	22.98	3.38	22.59	3.10	0.001
Household and recreational activities (MET-h/week)	36.41	24.43	39.82	26.28	<0.001
Age at menarche (years)	14.52	1.90	14.70	1.77	0.008
Age at first live birth (years)*	25.57	3.67	25.43	3.73	0.328
Total energy (kJ/d)	6050.06	1665.23	6108.64	1652.68	0.759
Total energy (kcal/d)	1446.42	398.06	1460.58	395.03	0.759
Total cruciferous vegetables (g/d)†	115.53	71.06	130.53	68.93	<0.001
Glucosinolate (mg/d)†	89.10	63.37	100.94	59.80	<0.001
Isothiocyanate ( $\mu$ mol/d)†	29.05	21.89	32.10	20.79	<0.001
	<i>n</i>	%	<i>n</i>	%	
Marital status					0.277
Married	1396	94.01	1401	93.03	
Unmarried/divorced/widowed	89	5.99	105	6.97	
Educational level					0.004
Primary school or below	375	25.25	388	25.76	
Junior high school	427	28.75	357	23.71	
Senior high school/secondary technical school	365	24.58	372	24.70	
College or above	318	21.41	389	25.83	
Occupation					0.316
Administrator/other white-collar worker	574	38.65	597	39.64	
Blue-collar worker	380	25.59	409	27.16	
Farmer/other	531	35.76	500	33.20	
Income (yuan/month)					0.015
<2000	300	20.20	248	16.47	
2001–5000	440	29.63	422	28.02	
5001–8000	392	26.40	442	29.35	
>8001	353	23.77	394	26.16	
Regular smoker	18	1.21	8	0.53	0.045
Passive smoker	837	56.36	756	50.20	0.001
Regular drinker	189	12.73	104	6.91	<0.001
Occupational activity					<0.001
Non-working	484	32.59	402	26.69	
Sedentary	478	32.19	490	32.54	
Standing	289	19.46	355	23.57	
Manual	132	8.89	170	11.29	
Heavy manual	102	6.87	89	5.91	
Menopausal status					0.245
Premenopausal	964	64.92	1008	66.93	
Postmenopausal	521	35.08	498	33.07	
Parity					0.172
0	63	4.24	72	4.78	
1–2	1085	73.06	1133	75.23	
≥3	337	22.69	301	19.99	
Breast-feeding*	1259	87.50	1291	87.82	0.824
First-degree relative with cancer	225	15.15	118	7.84	<0.001
Ever used an oral contraceptive	129	8.69	96	6.37	0.017

MET, metabolic equivalent task.

\* Among women who have had a live birth.

† The consumption was adjusted for total energy intake by the residual method.

quartile was 0.51 (95 % CI 0.41, 0.63) after adjusting for potential confounding factors ( $P_{\text{trend}} < 0.001$ ). Estimated GSL content exposure was also significantly and inversely related to the risk of breast

**Table 2.** Risks for breast cancer according to quartiles of cruciferous vegetables, glucosinolates (GSL) and isothiocyanates (ITC) intake (Odds ratios and 95 % confidence intervals)

	No. cases/controls	Crude OR	95 % CI	Adjusted OR*	95 % CI	<i>P</i> <sub>for trend</sub>
<b>Cruciferous vegetables (g/d)</b>						
<88-80	531/376	1.00		1.00		<0.001
88-80–121.06	378/377	0.71	0.59, 0.86	0.74	0.61, 0.91	
121.06–164.28	311/377	0.58	0.48, 0.71	0.62	0.50, 0.76	
≥164.28	265/376	0.50	0.41, 0.61	0.51	0.41, 0.63	
<b>GSL (mg/d)</b>						
<59.67	537/376	1		1		<0.001
59.67–89.78	341/376	0.64	0.52, 0.77	0.67	0.55, 0.82	
89.78–130.33	323/378	0.60	0.49, 0.73	0.62	0.51, 0.77	
≥130.33	284/376	0.53	0.43, 0.65	0.54	0.44, 0.67	
<b>ITC (μmol/d)</b>						
<17.59	483/376	1		1		<0.001
17.59–28.00	375/377	0.77	0.64, 0.94	0.83	0.67, 1.01	
28.00–43.01	341/377	0.70	0.58, 0.86	0.74	0.60, 0.92	
≥43.01	286/376	0.59	0.48, 0.73	0.62	0.50, 0.76	

\* OR adjusted for age, education, income, BMI, regular smoking, passive smoking, regular drinking, household and recreational activities, occupational activity, age at menarche, first-degree relative with cancer and ever used an oral contraceptive.

**Table 3.** Associations between quartiles of cruciferous vegetables, glucosinolate (GSL) and isothiocyanates (ITC) intake and breast cancer risk according to menopausal status (Odds ratios and 95 % confidence intervals)

	Quartile 1	Quartile 2		Quartile 3		Quartile 4		<i>P</i> <sub>for trend</sub>
		OR	95 % CI	OR	95 % CI	OR	95 % CI	
<b>Cruciferous vegetables (g/d)</b>								
<b>Premenopause</b>								
No. cases/controls	361/258	246/258		195/257		162/235		
Crude	1	0.68	0.54, 0.86	0.54	0.42, 0.69	0.49	0.38, 0.64	<0.001
Adjusted*	1	0.70	0.55, 0.90	0.60	0.46, 0.77	0.51	0.39, 0.67	<0.001
<b>Postmenopause</b>								
No. cases/controls	170/119	132/119		116/120		103/141		
Crude	1	0.77	0.55, 1.08	0.67	0.47, 0.95	0.51	0.36, 0.72	<0.001
Adjusted*	1	0.81	0.57, 1.16	0.63	0.44, 0.91	0.50	0.34, 0.72	<0.001
<b>GSL (mg/d)</b>								
<b>Premenopause</b>								
No. cases/controls	362/263	224/263		207/241		171/241		
Crude	1	0.62	0.49, 0.79	0.62	0.49, 0.80	0.52	0.40, 0.66	<0.001
Adjusted*	1	0.65	0.51, 0.84	0.66	0.51, 0.86	0.55	0.42, 0.72	<0.001
<b>Postmenopause</b>								
No. cases/controls	175/113	117/113		116/137		113/135		
Crude	1	0.67	0.47, 0.95	0.55	0.39, 0.77	0.54	0.38, 0.76	<0.001
Adjusted*	1	0.68	0.47, 0.98	0.52	0.36, 0.74	0.52	0.36, 0.75	<0.001
<b>ITC (μmol/d)</b>								
<b>Premenopause</b>								
No. cases/controls	316/280	253/250		220/236		175/242		
Crude	1	0.90	0.71, 1.14	0.83	0.65, 1.05	0.64	0.50, 0.83	0.001
Adjusted*	1	0.96	0.75, 1.23	0.89	0.69, 1.15	0.70	0.53, 0.91	0.010
<b>Postmenopause</b>								
No. cases/controls	167/96	122/127		121/141		111/134		
Crude	1	0.55	0.39, 0.79	0.49	0.35, 0.7	0.48	0.33, 0.68	<0.001
Adjusted*	1	0.55	0.37, 0.80	0.46	0.32, 0.67	0.45	0.31, 0.66	<0.001

\* OR adjusted for age, education, income, BMI, regular smoking passive smoker, regular drinking, household and recreational activities, occupational activity, age at menarche, first-degree relative with cancer and ever used an oral contraceptive.

cancer (highest *v.* lowest quartile: OR=0.54; 95 % CI 0.44, 0.67, *P*<sub>trend</sub><0.001). Similarly, women with high exposure of ITC had significantly lower risk of breast cancer (highest *v.* lowest quartile: adjusted OR=0.62; 95 % CI 0.50, 0.76, *P*<sub>trend</sub><0.001).

In all, there were 1972 premenopausal women (964 cases and 1008 controls) and 1019 postmenopausal women (521 cases and 498 controls). Stratified analysis by menopausal status (Table 3) showed a notably inverse association in both

premenopausal and postmenopausal women with high intakes of cruciferous vegetables. Compared with the lowest quartile, the adjusted OR in the highest quartile were 0.51 (95 % CI 0.41, 0.67, *P*<sub>trend</sub><0.001) among premenopausal women and 0.50 (95 % CI 0.34, 0.72, *P*<sub>trend</sub><0.001) among postmenopausal women. Analogously, the inverse associations between intakes of GSL, ITC and breast cancer risk were also observed in both premenopausal and postmenopausal women.

**Table 4.** Associations between quartiles of cruciferous vegetables, glucosinolate (GSL) and isothiocyanates (ITC) intake and breast cancer risk according to sex hormone receptor status (Odds ratios and 95% confidence intervals)

	Quartile 1	Quartile 2		Quartile 3		Quartile 4		<i>P</i> <sub>for trend</sub>
		OR	95% CI	OR	95% CI	OR	95% CI	
<b>Cruciferous vegetables (g/d)</b>								
<b>ER+ PR+</b>								
No. cases/controls	304/377	210/377		176/377		141/376		
Crude	1	0.69	0.55, 0.86	0.58	0.46, 0.73	0.46	0.36, 0.59	<0.001
Adjusted*	1	0.71	0.56, 0.90	0.61	0.48, 0.78	0.48	0.39, 0.62	<0.001
<b>ER+ PR-</b>								
No. cases/controls	28/377	19/377		15/377		16/376		
Crude	1	0.68	0.37, 1.23	0.53	0.28, 1.02	0.57	0.30, 1.07	0.050
Adjusted*	1	0.69	0.37, 1.27	0.57	0.30, 1.12	0.59	0.31, 1.14	0.082
<b>ER- PR+</b>								
No. cases/controls	29/377	20/377		14/377		17/376		
Crude	1	0.69	0.38, 1.24	0.48	0.25, 0.93	0.57	0.32, 1.09	0.042
Adjusted*	1	0.68	0.37, 1.23	0.53	0.28, 1.02	0.57	0.30, 1.07	0.224
<b>ER- PR-</b>								
No. cases/controls	80/377	59/377		36/377		38/376		
Crude	1	0.74	0.51, 1.06	0.45	0.30, 0.68	0.48	0.32, 0.72	<0.001
Adjusted*	1	0.73	0.50, 1.06	0.49	0.32, 0.76	0.50	0.33, 0.78	<0.001
<b>GSL (mg/d)</b>								
<b>ER+ PR+</b>								
No. cases/controls	307/376	183/376		178/378		163/376		
Crude	1	0.60	0.47, 0.75	0.58	0.46, 0.73	0.53	0.42, 0.67	<0.001
Adjusted*	1	0.63	0.50, 0.80	0.60	0.47, 0.76	0.55	0.43, 0.71	<0.001
<b>ER+ PR-</b>								
No. cases/controls	29/377	15/377		20/377		14/376		
Crude	1	0.52	0.23, 0.98	0.69	0.38, 1.23	0.48	0.25, 0.93	0.049
Adjusted*	1	0.53	0.27, 1.01	0.72	0.39, 1.32	0.51	0.26, 1.00	0.612
<b>ER- PR+</b>								
No. cases/controls	28/377	20/377		12/377		17/376		
Crude	1	0.71	0.40, 1.29	0.53	0.28, 1.01	0.61	0.33, 1.13	0.065
Adjusted*	1	0.76	0.41, 1.40	0.60	0.31, 1.18	0.78	0.41, 1.50	0.316
<b>ER- PR-</b>								
No. cases/controls	75/376	65/376		39/378		34/376		
Crude	1	0.87	0.60, 1.24	0.52	0.34, 0.78	0.45	0.30, 0.70	<0.001
Adjusted*	1	0.84	0.58, 1.23	0.53	0.35, 0.82	0.48	0.30, 0.75	<0.001
<b>ITC (μmol/d)</b>								
<b>ER+ PR+</b>								
No. cases/controls	275/376	206/377		184/377		166/376		
Crude	1	0.75	0.59, 0.94	0.67	0.53, 0.84	0.60	0.48, 0.77	<0.001
Adjusted*	1	0.80	0.63, 1.01	0.71	0.60, 0.91	0.64	0.50, 0.82	<0.001
<b>ER+ PR-</b>								
No. cases/controls	29/376	13/377		19/377		17/376		
Crude	1	0.45	0.23, 0.87	0.65	0.36, 1.19	0.59	0.32, 1.09	0.141
Adjusted*	1	0.50	0.25, 0.98	0.71	0.38, 1.32	0.65	0.34, 1.24	0.253
<b>ER- PR+</b>								
No. cases/controls	23/377	28/377		12/377		17/376		
Crude	1	1.12	0.69, 2.15	0.52	0.26, 1.06	0.74	0.39, 1.41	0.099
Adjusted*	1	1.29	0.71, 2.33	0.61	0.29, 1.27	0.94	0.48, 1.85	0.412
<b>ER- PR-</b>								
No. cases/controls	63/376	69/377		46/377		35/376		
Crude	1	1.09	0.75, 1.58	0.73	0.49, 1.09	0.56	0.36, 0.86	0.002
Adjusted*	1	1.09	0.74, 1.60	0.76	0.49, 1.16	0.61	0.38, 0.96	0.013

ER+, oestrogen receptor-positive; ER-, oestrogen receptor negative; PR+, progesterone receptor positive; PR-, progesterone receptor negative.  
 \* OR adjusted for age, education, income, BMI, regular smoking, passive smoking, regular drinking, household and recreational activities, occupational activity, age at menarche, first-degree relative with cancer and ever used an oral contraceptive.

When cases were grouped on the basis of sex hormone receptor status, 831 cases were in ER+ PR+ status, seventy-eight cases in ER+ PR- status, eighty cases in ER- PR+ status and 213 cases in ER- PR- status. Among cases with ER+ PR+ and ER- PR- status, intakes of cruciferous vegetables, GSL and ITC were inversely associated with breast cancer risk. For the ER+ PR- and ER- PR+ subtype, a non-significant inverse association

was found between cruciferous vegetables, GSL, ITC and breast cancer risk (Table 4).

### Discussion

Our case-control study among Chinese women revealed an inverse association between cruciferous vegetable intake and

breast cancer risk. Estimated GSL and ITC content exposure was also inversely associated with the risk of breast cancer.

In the present study, ten types of cruciferous vegetables including choy sum, kaichoi, kailan, broccoli, pakchoi, bokchoi, cabbage, mini Chinese cabbage, cauliflower and radish common in Guangdong province were included. The inverse association between cruciferous vegetable consumption and breast cancer risk observed in the present study was in agreement with the meta-analysis published in 2013<sup>(10)</sup>. However, no statistically significant association between cruciferous vegetable intake and breast cancer risk was found in most studies conducted in Western populations<sup>(28–35)</sup>. Some possible explanations might account for the different results. First, the level of cruciferous vegetable consumption differed among different populations. The energy-adjusted median intake of cruciferous vegetables in our population was 130 g/d, which was >3 g/d in Japanese women<sup>(30)</sup>, 11.3 g/d in the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC)<sup>(36)</sup> and 31 g/d in women from the Nurses' Health Study<sup>(37)</sup>. The lack of association between consumption of cruciferous vegetables and breast cancer risk in Western populations might be owing to their much lower level of consumption. Second, owing to the difference of dietary patterns between China and Western countries<sup>(38)</sup>, the types of cruciferous vegetables consumed varied in different countries. For example, broccoli, cauliflower, cabbage, collard green and Brussels sprout are commonly consumed in Western countries, whereas choy sum, kaichoi, kailan, pakchoi and bokchoi are mostly consumed in Asian diets<sup>(39)</sup>.

To our knowledge, no epidemiological study has yet been published to examine the association between GSL intake and breast cancer risk. Only the EPIC cohort<sup>(40)</sup>, which is a multi-centre prospective cohort study comprising 11 405 men, assessed the relationship between dietary GSL intake and prostate cancer risk. A significant inverse association was found between GSL intake and prostate cancer risk, with an adjusted hazard ratios of 0.68 (95% CI 0.48, 0.97) comparing the highest with the lowest quartile. In this study, a notable 46% reduction of breast cancer risk was observed among women with a high intake of the GSL obtained from the cruciferous vegetables commonly consumed in China.

As a vital bioactive breakdown component of GSL, the association between ITC intake and breast cancer has been investigated in only a few epidemiological studies. Consistent with the result of the Shanghai Breast Cancer Study<sup>(41)</sup>, our result suggested a 38% reduction in breast cancer risk with ITC intake. Besides, two population-based case-control studies found an inverse association between ITC and lung cancer risk in both African-American and Caucasian women from America<sup>(16)</sup>, and Chinese women from Singapore<sup>(17)</sup>. However, no significant association between ITC intake and oral cancer risk was found in a hospital-based case-control study consisting of 115 oral cancer cases and 116 controls conducted in Malaysia<sup>(42)</sup>.

Cruciferous vegetables contain numerous nutrients and phytochemicals with cancer chemo-preventive properties, including carotenoids, folate, fibre and chlorophyll<sup>(12)</sup>. Furthermore, what makes cruciferous vegetables unique are rich

sources of GSL. Nearly 120 GSL with unique breakdown products have been identified in cruciferous vegetables<sup>(43)</sup>. Owing to the chemical and thermal stability of most GSL, the hydrolysis of GSL relies on plant myrosinase, which exists physically separated from GSL in plant cells. When intact cruciferous vegetables are chopped or chewed, GSL come in contact with plant myrosinase, resulting in the formation of ITC at a neutral pH of 6–7<sup>(44)</sup>. ITC exert their chemoprotective effect by two major mechanisms. One mechanism is inhibiting the biotransformation of chemical carcinogens by specific CYP enzymes to active forms that are capable of binding DNA to induce mutations. The other mechanism is that ITC potentially induced phase II enzyme activity, primarily GST, by increasing the transcription of genes that contain an antioxidant response element<sup>(45)</sup>. ITC are water-soluble compounds that may be leached into cooking water, and thus we estimated dietary ITC intake by using previously published ITC concentrations for cruciferous vegetables cooked in boiling water<sup>(25)</sup>.

Stratified analysis by menopausal status found no disparity between premenopausal and postmenopausal women. Our findings are also consistent with those of an intervention study<sup>(46)</sup>, which showed that the addition of 500 g/d brassica to the daily diet of thirty-four healthy postmenopausal women may shift oestrogen metabolism, as measured by the increased values of the biomarker ratio 2-hydroxyoestrone:16 $\alpha$ -hydroxyoestrone in urine. Compared with 2-hydroxyoestrone, 16 $\alpha$ -hydroxyoestrone is relatively more oestrogenic and genotoxic. Therefore, this change in oestrogen metabolism may be a mechanism to protect against breast cancer<sup>(47)</sup>. Similar results were found in another small sample intervention study<sup>(48)</sup>, which reported that eleven of the thirteen premenopausal women showed strongly positive increases in their 2-hydroxyoestrone:16 $\alpha$ -hydroxyoestrone ratios in urine after using the supplements of dried cruciferous powder for 90 d. However, a recent meta-analysis of eleven case-control studies and two cohort studies examining the associations between cruciferous vegetables and breast cancer risk<sup>(10)</sup> showed that the protective effect of cruciferous vegetables against breast cancer risk was present only in postmenopausal women. As there were only five studies in the premenopausal group and the heterogeneity was high ( $P=0.004$ ), the results are still inconclusive. More studies are needed to clarify this association of cruciferous vegetables and breast cancer risk defined by menopausal status.

In this study, higher consumption of cruciferous vegetables, GSL and ITC was found to be inversely associated with breast cancer risk in women with both hormone-receptor-positive or hormone-receptor-negative status. Consistent with our results, a cohort study conducted in Japan<sup>(30)</sup> reported that cruciferous vegetable intake was marginally inversely associated with ER+ and PR+ tumours. The adjusted relative risk was 0.64 (95% CI 0.41, 1.00), comparing the highest with the lowest quartile. The Pooling Project of Prospective Studies of Diet and Cancer, comprising twenty cohort studies<sup>(49)</sup>, indicated that total vegetable consumption was significantly and inversely associated with the risk of ER- breast cancer (highest *v.* lowest quintile: adjusted OR = 0.82; 95% CI 0.74, 0.90). Similar result was found in EPIC cohort study<sup>(50)</sup> and the Nurses' Health Study<sup>(51)</sup>. The rational biological mechanism is that ITC can affect hormonal



level through inhibiting the transcription of oestrogen-simulated target genes such as ER and PR, thus preventing the development of ER+ PR+ tumours<sup>(52)</sup>. The mechanism for lowering ER- tumours might be hormonally independent. It was reported that ER- tumours are more likely to be less dependent on oestrogen levels than ER+ tumours<sup>(52)</sup>. Studies showed that NF- $\kappa$ B, epidermal growth factor receptor and cyclin E were over-expressed in ER- breast tumours, leading to the development of breast cancer. Bioactive components contained in cruciferous vegetables might reduce the levels of these factors<sup>(53)</sup> and lower the risk of ER- breast cancer. Our results showed a non-significant inverse association among the ER+ PR- and ER- PR+ subtypes. However, as the cases in the subgroups are relatively small, further studies are needed to clarify this issue.

The strengths of the present study include the relatively large sample size, and the satisfactory reproducibility and reasonable validity of the FFQ. In addition, this is the first study to evaluate the association between cruciferous vegetable intakes and breast cancer risk according to GSL and ITC contents. Our attempt to measure GSL and ITC intake based on various cruciferous vegetables commonly consumed locally further strengthen the study findings.

The study has a few limitations. First, the results could be affected by recall bias and selection bias, which are inevitable in hospital-based case-control studies. To minimise selection bias, control subjects were recruited to exclude any disease potentially related either to breast cancer or dietary changes. However, the time-concordant period of hospitalisation, identical catchment areas of all subjects and the relatively high response rate helped to reduce the selection bias. Moreover, to minimise recall bias, cases were interviewed as soon as the diagnosis was made and as far as possible before their surgery. In addition, food photographs were provided to assist participants with quantification of dietary intake. Second, residual confounding remained even though various dietary and non-dietary confounders were adjusted in the multivariable models. Third, this study did not measure urinary ITC, which have been shown to be useful biomarkers for recent cruciferous vegetable intake<sup>(54,55)</sup>. Further studies are needed to assess the association between cruciferous vegetable intake and breast cancer risk by measuring urinary ITC.

In conclusion, this study indicated that higher cruciferous vegetables, GSL and ITC intakes were inversely associated with breast cancer risk among Chinese women. The findings suggested the protective role of cruciferous vegetables, rich in GSL and ITC, against breast cancer.

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The authors' responsibilities were as follows: N.-Q. Z. conducted the data collection, analysed the data and writing of this paper. S. C. H. provided significant advice regarding the

analyses and interpretation of the data. W.-Q. H., H. L. and J. H., participated in the data collection and data entry. X.-F. M. and F.-Y. L. were responsible for connecting and coordinating the field work. C.-X. Z. constructed the project design, and supervised and contributed to manuscript writing.

The authors declare that there are no conflicts of interest.

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