Association between phytosterol intake and colorectal cancer risk: a case–control study

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Abstract

A study in rodent models showed that phytosterols protected against colon carcinogenesis, probably by inhibiting dysregulated cell cycle progression and inducing cellular apoptosis. However, epidemiological studies on the relationship between phytosterols and colorectal cancer risk are quite limited. The aim of this study was to investigate dietary phytosterol intake in relation to colorectal cancer risk in the Chinese population. A case–control study was conducted from July 2010 to June 2016, recruiting 1802 eligible colorectal cancer cases plus 1813 age (5-year interval) and sex frequency-matched controls. Dietary information was collected by using a validated FFQ. The OR and 95 % CI of colorectal cancer risk were assessed by multivariable logistic regression models. A higher total intake of phytosterols was found to be associated with a 50 % reduction in colorectal cancer risk. After adjusting for various confounders, the OR of the highest quartile intake compared with the lowest quartile intake was 0·50 (95 % CI 0·41, 0·61, $P_{trend} < 0·01$) for total phytosterols. An inverse association was also found between the consumption of β-sitosterol, campesterol, campestanol and colorectal cancer risk. However, stigmasterol intake was related to an increased risk of colorectal cancer. No statistically significant association was found between β-sitostanol and colorectal cancer risk. Stratified analysis by sex showed that the positive association of stigmasterol intake with colorectal cancer risk was found only in women. These data indicated that the consumption of total phytosterols, β-sitosterol, campesterol and campestanol is inversely associated with colorectal cancer risk in a Chinese population.

Key words: Phytosterols: Colorectal cancer risk: Case–control studies: China

Growing numbers of studies have revealed that vegetable and fruit consumption is inversely associated with colorectal cancer risk[1]. Concomitantly, there has been increasing interest in phytochemicals[2,3], such as flavonoids[4], polyphenols[5] and phytosterols[6], which could be responsible for the protective effect of vegetable and fruit against colorectal cancer. Phytosterols are derivatives of the parent molecule 4-desmethyl sterol and are found exclusively in plant food. They are 28- or 29-carbon alcohols that structurally resemble 27-carbon cholesterol[7]. The most common phytosterols in the human diet are β-sitosterol, campesterol, stigmasterol. All phytosterols contain a double bond at carbon–5; when this double bond is saturated under certain conditions, these compounds are referred to as plant stanols, the two most common of which are β-sitostanol and campestanol[8]. Oils, nuts, legumes, cereals, vegetables and fruits are the main dietary sources of phytosterols[9,10].

Regarding their bioactivity, phytosterols have been proposed not only to lower serum cholesterol levels[11] but also to reduce cancer risk by mediating cell cycle arrest, inducing cellular apoptosis[12] and reducing cellular oxidative stress[13], all of which have been experimentally verified. Moreover, epidemiological studies have reported that phytosterol consumption could be beneficial in reducing the risk of cancer in the lung[14], breast[15], oesophagus[16], stomach[17] and ovaries[18].

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Regarding the anticancer effect of phytosterols on colorectal cancer, Nair *et al.*[19] found that Seventh-day Adventists who consumed high levels of phytosterols in their diets had a decreased risk of developing colorectal cancer compared with the general population. On the other hand, a case–cohort study conducted in the Netherlands found no reduction in colorectal cancer risk in relation to a high intake of any phytosterol[20].

It was reported that total vegetable and fruit consumption was 440 g/d among men and 502 g/d among women in the Chinese population[21], which is higher than the consumption of people from European countries (397 g/d)[22]. To date, no study has focused on the relationship between the intake of plant-origin nutrients, phytosterols and colorectal cancer risk in the Chinese population. Therefore, the present work aims to examine this issue. As a previous investigation concluded that a high consumption of vegetables and fruit is negatively associated with colorectal cancer[23], we hypothesised that a higher intake of phytosterols from plant foods could decrease the risk of colorectal cancer.

Methods

Study subjects

This is an ongoing case–control study that began in July 2010. Potential case subjects were consecutively recruited from patients admitted to the surgical units of the Sun Yat-sen University Cancer Center, Guangzhou, China. Eligible cases were required to be patients with incident, primary, histologically confirmed colorectal cancer diagnosed no more than 3 months before the interview, aged 30–75 years, and that either were natives of Guangdong province or had lived in Guangdong for at least 5 years. Subjects were excluded if they could not understand or speak Mandarin/Cantonese or had a history of any cancer. Furthermore, subjects with an energy intake that was too low or too high (<2510 or >14 644 kJ/d (<600 or >3500 kcal/d) for women, <3347 or >17 573 kJ/d (<800 or >4200 kcal/d) for men)[24] were not included in the analysis. From July 2010 to June 2016, 2012 eligible cases were identified and 1805 were successfully interviewed, with a response rate of 89·71 %; 207 patients did not complete the investigation, mainly due to fatigue, communication barriers and refusal. Three subjects with an energy intake that was too low or too high were not included in the analysis. Finally, 1802 cases were included in the analysis.

Two control groups were used in this case–control study. The first control group was recruited from the in-patients admitted to three affiliated hospitals of the Sun Yat-sen University during the same period, with eye disorders, ear–nose–throat diseases, trigeminal neuralgia, varicose veins, osteoarthritis, degenerate joint disease, orthopaedics, facial paralysis and acute appendicitis. The second control group was recruited from residents of the same community via advertisements, written invitations or referrals. The eligibility criteria for the controls were the same as described for the cases except that they had no history of colorectal cancer. They were frequency-matched by age (5-year interval) and sex to the cases. In total, 1059 hospital-derived controls were identified and 924 were successfully interviewed

in the study, yielding a participation rate of 87·25 %. In addition, 889 community-derived controls were interviewed.

We assumed that people with higher phytosterols intake was 25 % among the general population, the estimated OR between phytosterols intake and colorectal cancer risk was 0·67[20], the significant *P* value was <0·05 ($\alpha = 0·05$), the power of test was 90 % ($\beta = 0·1$), and the response rate was 80 %, thus leading to the calculated sample size of 979 cases.

This 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. All the participants signed informed consent forms before the interview.

Data collection

Both cases and controls were interviewed face-to-face by trained interviewers using a structured questionnaire that collected information on socio-demographic characteristics, family history of cancer, lifestyle (e.g. active and passive smoking, alcohol consumption and physical activity) and body measurements (weight and height). For female subjects, menstrual and reproductive histories were also obtained. Relevant medical data such as diagnosis and histological findings were extracted from the hospital medical records. In this study, smokers were defined as people who had smoked at least one cigarette a day for more than 6 months consecutively or accumulatively in their lifetime[25]. Passive smokers were defined as non-smokers who reported being exposed to the smoke exhaled by smokers for at least 15 min/d over a week. Regular drinking was defined as the consumption of alcohol at least once per week during the past year. The level of physical activity was assessed based on self-reported occupational, household and leisure activity in the previous year. Frequency (d/week) and duration (h/d) data were collected for household and leisure activity. According to labour intensity, occupational activity was classified as follows: (a) not working or retired; (b) long time sitting; (c) low intensity; (d) moderate intensity; or (e) vigorous intensity, with examples provided. Household and entertainment activities were combined in this study. They were categorised into light physical activity (walking, etc.), moderate physical activity (mountaineering, jogging, playing table tennis, etc.) and vigorous physical activity (running, playing football/basketball, etc.). The mean metabolic equivalent task (MET)-hours value of each activity was acquired by estimating the average of all comparable activities in the Compendium of Physical Activities[26,27]. MET-hours per week over the past 12 months was calculated as follows: number of days per week × number of hours per day × MET for a specific type of activity = MET-hours per week. Postmenopausal status was defined as at least 12 months since the last menstrual cycle. BMI was calculated as the ratio of weight (kg) to squared height (m^2).

The subjects completed an interviewer-administered FFQ that included eighty-one food items and assessed their dietary habits. The main food groups included cereal products, legumes, vegetables, fruits, red and processed meat, poultry, fish and other seafood, egg, dairy products and nuts. Dietary information was requested from the cases covering the year

before diagnosis and from controls covering the year before interview. The participants were asked to report how often they consumed each food and the average amount eaten each time. Food photographs were used to help participants to quantify the portions consumed. Information on cooking oil intake was also collected. The subjects were asked to report how much cooking oil was consumed by the whole family in 1 month, the number of family members and which kind of oils they preferred to use (such as peanut oil, rapeseed oil, maize oil and blend oil). Then the quantity of cooking oil for each subject consumed per day was calculated. Phytosterol intake was estimated by summing the amount of food consumed and the phytosterol content, which was estimated according to a previous report in China[10]. In total, five frequently occurring phytosterols were measured: the unsaturated plant sterols β-sitosterol, campesterol and stigmasterol, and the saturated plant stanols β-sitostanol and campestanol. In the present study, all plant-derived foods were considered to be sources of phytosterols, including plant oils, cereal products, legumes, nuts, vegetables and fruits. The 2002 Chinese Food Composition Table was used to calculate energy and other nutrient intakes[28]. As part of the study, we also collected information on whether study participants had changed their dietary habits in the past 5 year before the interview. Study participants were considered as having significant dietary changes if they reported 'Yes' to the following questions: 'Compared with the previous years, have you changed your dietary habits in the past five year?'

The FFQ has been validated and tested for reproducibility[29], and has been used in previous studies[23]. The energy-adjusted Pearson's correlation coefficients comparing the FFQ and the six 3-d dietary records were 0·25–0·65 for nutrients and 0·30–0·68 for food groups. The correlation coefficients for the reproducibility of the FFQ were 0·46–0·71 for nutrients and 0·36–0·66 for food groups.

Statistical analysis

SPSS 21.0 (SPSS Inc.) was used to conduct the data analysis. The χ^2 test was used to test the difference between the cases and controls for the categorical variables, and the t test or Wilcoxon's signed-rank test was used for the continuous variables. Dietary phytosterol intake was adjusted based on total energy intake of men and women, using the regression residual method[30]. Phytosterol consumption was categorised into quartiles (Q1–Q4) based on the distribution among controls for men and women separately. Unconditional logistic regression models were used to estimate the OR and 95 % CI for the associations between phytosterol intake and colorectal cancer risk, using the lowest quartile as the reference group. According to the comparison of the characteristics of the cases and controls or previous reported confounders, the following variables were selected for multivariable-adjusted models: age, sex, marital status, residence, education, occupation, income level, BMI, smoking status, passive smoking, alcohol drinking, family history of cancer, occupational physical activity, household and leisure-time activities, intake of dairy products, red and processed meat. All confounding factors were included as categorical variables except for age, BMI, household and

leisure-time activities, intake of dairy products, red and processed meat which were treated as continuous variables. Consumption of phytosterols, dairy products, red and processed meat in the multivariable model was adjusted for energy intake according to the residual method. Tests for trend were performed by entering the categorical variables as continuous variables in the multiple regression models.

Stratified analysis by sex was conducted. The interaction effect between phytosterols intake and sex was performed. Interaction terms were calculated as the products of the stratified factor (sex, dichotomous) and the phytosterols intake (continuous). Subgroup analysis by cancer site (colon or rectal cancer) and by sources of controls (community-derived controls and hospital-derived controls) was conducted. Sensitivity analysis was also performed by excluding study subjects who had dietary changes during the past 5 years, because of the possibility that pre-diagnosis symptoms might alter the dietary intake. In addition, the Pearson's correlation coefficient between intakes of total phytosterols and total dietary fibre was calculated. In this study, all P values were two-sided and a P value <0·05 was deemed to denote statistical significance.

In the present study, the sample size of 1003 cases and 906 controls in two quartiles (Q1 and Q4) gave us greater than 99 % power to detect the OR of 0·52 for the association between total phytosterols intake and colorectal cancer risk. We also had greater than 99 % power to detect the OR of 0·48, 0·43 and 0·60 for β-sitosterol, campesterol and campestanol, respectively. However, the power was less than 65 % for stigmasterol and β-sitostanol.

Results

Among the 1802 cases, 1011 were men and 791 were women. In total, 1091 were diagnosed with colon cancer and 711 with rectal cancer. The distribution of their socio-demographic and several relevant characteristics are shown in Table 1. Compared with the controls, more cases lived in rural area, were less educated, had higher incomes, had a higher frequency of smoking, passive smoking and regular drinking experiences, engaged in more occupational activities, engaged in fewer household and leisure activities and tended to have a family history of cancer. No significant differences were found between the case and control subjects in age, sex, marital status, occupation, BMI, age at menarche and menopausal status.

The total intake of five phytosterols was 202·6 (SD 76·3) mg/d for cases and 225·9 (SD 79·2) mg/d for controls; the difference between these values was statistically significant. Compared with the controls, the cases had a higher intake of red and processed meat, and a lower intake of total energy, β-sitosterol, campesterol, campestanol and dairy products. No significant difference was found in dietary intake of stigmasterol and β-sitostanol between cases and controls (Table 2).

The OR and 95 % CI of colorectal cancer for phytosterols are shown in Table 3. For total phytosterols, the highest quartile intake showed a risk reduction of 50 % compared with the lowest quartile (OR 0·50; 95 % CI 0·41, 0·61, P_{trend} <0·01). For the specific phytosterols, after controlling for the potential

Table 1. Demographic and selected risk factors of colorectal cancer cases and controls in Chinese population*
(Mean values and standard deviations; medians and 25th, 75th percentiles)

	Cases (*n* 1802)		Controls (*n* 1813)		
	n	%	*n*	%	*P*
Age (years)					0·15
Mean	57·1		56·2		
SD	25·5		9·7		
Sex					0·97
Male	1011	56·1	1016	56·0	
Female	791	43·9	797	44·0	
Marital status					0·06
Married	1708	94·8	1691	93·3	
Unmarried/divorced/widowed	94	5·2	122	6·7	
Residence					0·01
Urban	1146	63·6	1229	67·8	
Rural	656	36·4	584	32·2	
Educational level					<0·01
Primary school or below	589	32·7	385	21·2	
Secondary school	488	27·1	472	26·1	
High school	423	23·5	530	29·2	
College or above	302	16·8	426	23·5	
Occupation					0·05
Administrator/other white-collar worker	245	13·6	275	15·2	
Blue-collar worker	362	20·1	407	22·4	
Farmer/other	1195	66·3	1131	62·4	
Income (yuan/month)					<0·01
<2000	270	15·0	286	15·8	
2001–5000	557	30·9	651	36·0	
5001–8000	515	28·6	553	30·6	
>8001	460	25·5	316	17·5	
BMI (kg/m^2)					0·24
Mean	23·2		23·3		
SD	3·2		3·1		
Smokers	699	38·8	572	31·5	<0·01
Passive smoking	842	46·7	593	32·7	<0·01
Regular drinker	318	17·6	243	13·4	<0·01
Family history of cancer	247	13·7	148	8·2	<0·01
Occupational activity					<0·01
Non-working	654	36·3	865	47·7	
Sedentary	344	19·1	288	15·9	
Light occupation	319	17·7	380	21·0	
Moderate occupation	241	13·4	172	9·5	
Heavy activity occupation	244	13·5	107	5·9	
Household and leisure-time activities (MET-h/week)					<0·01
Median	28·9		36·0		
25th, 75th percentiles	8·8, 52·5		19·0, 57·2		
Age at menarche (years)†					0·33
Mean	14·6		14·5		
SD	2·4		2·6		
Menopausal status†					0·99
Premenopausal	228	28·8	230	28·9	
Postmenopausal	563	71·2	567	71·1	

MET, metabolic equivalent task.
* Continuous variables were evaluated using *t* tests or Wilcoxon rank-sum tests. Categorical variables were evaluated using χ^2 tests.
† Among female subgroup.

confounders, the OR of the highest quartile intake compared with the lowest quartile intake were 0·46 (95 % CI 0·37, 0·57, $P_{\text{trend}} < 0·01$) for β-sitosterol, 0·42 (95 % CI 0·34, 0·52, $P_{\text{trend}} < 0·01$) for campesterol and 0·67 (95 % CI 0·54, 0·83, $P_{\text{trend}} < 0·01$) for campestanol. However, stigmasterol was found to be associated with an increased risk of colorectal cancer, with an adjusted OR of 1·26 (95 % CI 1·03, 1·54, $P_{\text{trend}} = 0·01$) comparing the highest with the lowest quartile. No statistically significant association was found between β-sitostanol intake and colorectal cancer risk.

Stratified analysis by sex showed that the intake of total phytosterols, β-sitosterol and campesterol was inversely associated with colorectal cancer risk in both sexes, whereas no significant association was found between β-sitostanol intake and colorectal cancer risk neither in men nor women. Stigmasterol intake was found to be positively associated with colorectal cancer risk only among women. Compared with the lowest quartile, the highest quartile had an adjusted OR of 1·50 (95 % CI 1·11, 2·03, $P_{\text{trend}} < 0·01$). No significant association was found between stigmasterol intake and colorectal cancer

Table 2. Intakes of energy, phytosterol and foods among case and control subjects in Guangdong, China*
(Mean values and standard deviations; medians and 25th, 75th percentiles)

	Cases (n 1802)				Controls (n 1813)				
	Mean	SD	Median	25th, 75th percentiles	Mean	SD	Median	25th, 75th percentiles	P
Energy (kJ/d)	6740	2073·1	6452	5263, 7858	7276	2257·2	6979	5661, 8502	
Energy (kcal/d)	1611	495·5	1542	1258, 1878	1739	539·5	1668	1353, 2032	<0·01
Total phytosterols (mg/d)†	202·6	76·3	190·54	153·35, 236·28	225·9	79·2	212·79	172·82, 261·33	<0·01
β-Sitosterol (mg/d)†	127·6	49·5	120·54	95·28, 149·62	144·6	51·7	135·72	110·26, 167·81	<0·01
Campesterol (mg/d)†	30·7	15·3	26·64	21·08, 34·53	36·5	16·8	31·76	23·93, 46·24	<0·01
Stigmasterol (mg/d)†	23·7	7·1	23·09	18·92, 27·86	23·5	6·9	22·44	18·68, 26·96	0·05
β-Sitostanol (mg/d)†	17·7	7·1	16·81	13·74, 19·88	18·1	7·6	16·39	13·91, 19·74	0·98
Campestanol (mg/d)†	3·2	1·0	3·08	2·59, 3·66	3·5	1·1	3·33	2·75, 3·98	<0·01
Dairy products (g/d)†	52·8	172·8	0·27	0, 29·42	94·5	232·8	18·39	0·23, 89·70	<0·01
Red and processed meat (g/d)†	130·0	68·4	120·16	81·88, 166·19	99·0	54·9	90·45	58·50, 129·16	<0·01

* Wilcoxon's rank-sum test comparing the median consumption levels between cases and controls.
† The consumption was adjusted for total energy intake by the regression residual method.

Table 3. OR of colorectal cancer according to quartiles (Q) of phytosterol intake
(Odds ratios and 95 % confidence intervals)

	Q1	Q2		Q3		Q4		
	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}
Total phytosterols								
Median intake (mg/d)	154·8	192·7		235·1		294·5		
No. of cases/controls	695/453	447/453		352/454		308/453		
Crude	1	0·64	0·54, 0·77	0·51	0·42, 0·61	0·44	0·37, 0·53	<0·01
Adjusted*	1	0·67	0·55, 0·81	0·54	0·44, 0·66	0·50	0·41, 0·61	<0·01
β-Sitosterol								
Median intake (mg/d)	97·4	123·1		151·3		189·9		
No. of cases/controls	716/453	439/453		352/454		295/453		
Crude	1	0·61	0·51, 0·73	0·49	0·41, 0·59	0·41	0·34, 0·50	<0·01
Adjusted*	1	0·64	0·53, 0·78	0·54	0·44, 0·66	0·46	0·37, 0·57	<0·01
Campesterol								
Median intake (mg/d)	20·8	27·4		37·9		57·0		
No. of cases/controls	688/453	551/453		302/454		261/453		
Crude	1	0·80	0·67, 0·95	0·44	0·36, 0·53	0·38	0·31, 0·46	<0·01
Adjusted*	1	0·86	0·71, 1·03	0·49	0·40, 0·60	0·42	0·34, 0·52	<0·01
Stigmasterol								
Median intake (mg/d)	16·5	20·6		24·5		30·6		
No. of cases/controls	444/453	394/454		428/453		536/453		
Crude	1	0·89	0·73, 1·07	0·96	0·80, 1·16	1·21	1·01, 1·45	0·02
Adjusted*	1	0·83	0·67, 1·02	0·94	0·76, 1·15	1·26	1·03, 1·54	0·01
β-Sitostanol								
Median intake (mg/d)	12·5	15·1		17·7		23·7		
No. of cases/controls	477/453	352/454		482/453		491/453		
Crude	1	0·74	0·61, 0·89	1·01	0·84, 1·21	1·03	0·86, 1·23	0·20
Adjusted*	1	0·74	0·60, 0·91	1·05	0·86, 1·29	1·20	0·98, 1·47	0·01
Campestanol								
Median intake (mg/d)	2·4	3·2		3·7		4·6		
No. of cases/controls	643/453	474/454		386/453		299/453		
Crude	1	0·74	0·62, 0·88	0·60	0·50, 0·72	0·47	0·39, 0·56	<0·01
Adjusted*	1	0·86	0·71, 1·05	0·79	0·64, 0·97	0·67	0·54, 0·83	<0·01

* OR was adjusted for age, sex, marital status, residence, education, occupation, income level, BMI, smoking status, passive smoking, alcohol drinking, family history of cancer, occupational physical activity, household and leisure-time activities, dietary intake of dairy products, red and processed meat.

risk among men. Campestanol intake was found to be related to a decreased risk of colorectal cancer only among men (Table 4).

The results of the subgroup analysis by cancer site were similar to the main analysis in all colorectal cancer cases. The consumption of total phytosterols, β-sitosterol, campesterol and campestanol appeared to be inversely associated with the risk of both colon cancer and rectal cancer. For the intake of stigmasterol and β-sitostanol, both of them were observed to be related to an increased risk of colon cancer. Compared with the lowest quartile, the highest quartile had an adjusted OR of 1·41 (95 % CI 1·12, 1·79, P_{trend} < 0·01) and 1·35 (95 % CI 1·07, 1·71, P_{trend} < 0·01), respectively. However, no significant association was found between the consumption of stigmasterol or β-sitostanol and the risk of rectal cancer (Table 5). Furthermore, subgroup analysis by community-derived controls and hospital-derived controls was conducted. The inverse associations

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Table 4. OR of colorectal cancer according to quartiles (Q) of phytosterol intake by sex
(Odds ratios and 95 % confidence intervals)

	Men (n 1011)									Women (n 791)									
	Q1	Q2		Q3		Q4			Q1	Q2		Q3		Q4			
	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}	$P_{interaction}$
Total phytosterols																	
Median intake (mg/d)	162·1	206·9		248·8		305·6			146·5	177·6		215·0		281·6			
No. of cases/controls	425/254	257/254		169/254		160/254			270/199	190/199		183/200		148/199			
Crude	1	0·61	0·48, 0·76	0·40	0·31, 0·51	0·38	0·29, 0·48	<0·01	1	0·70	0·54, 0·92	0·67	0·51, 0·89	0·55	0·41, 0·73	<0·01	
Adjusted*	1	0·61	0·47, 0·81	0·39	0·30, 0·53	0·40	0·30, 0·55	<0·01	1	0·74	0·55, 0·99	0·74	0·55, 1·00	0·61	0·45, 0·83	<0·01	0·03
β-Sitosterol																	
Median intake (mg/d)	101·0	130·6		158·1		195·4			94·2	114·5		140·2		181·9			
No. of cases/controls	431/254	258/254		175/254		147/254			285/199	181/199		177/200		148/199			
Crude	1	0·60	0·47, 0·76	0·41	0·32, 0·52	0·34	0·26, 0·44	<0·01	1	0·64	0·48, 0·83	0·62	0·47, 0·81	0·52	0·39, 0·69	<0·01	
Adjusted*	1	0·60	0·45, 0·78	0·42	0·31, 0·55	0·35	0·26, 0·48	<0·01	1	0·71	0·53, 0·95	0·71	0·53, 0·95	0·60	0·44, 0·81	<0·01	0·02
Campesterol																	
Median intake (mg/d)	22·3	29·7		41·1		59·2			19·5	24·7		33·9		51·2			
No. of cases/controls	426/254	321/254		137/254		127/254			262/199	230/199		165/200		134/199			
Crude	1	0·75	0·60, 0·95	0·32	0·25, 0·42	0·30	0·23, 0·39	<0·01	1	0·88	0·67, 1·14	0·63	0·48, 0·83	0·51	0·38, 0·68	<0·01	
Adjusted*	1	0·78	0·60, 1·02	0·35	0·26, 0·47	0·31	0·23, 0·42	<0·01	1	0·92	0·69, 1·23	0·70	0·51, 0·94	0·58	0·43, 0·80	<0·01	<0·01
Stigmasterol																	
Median intake (mg/d)	17·3	21·6		26·0		32·7			15·6	19·5		22·7		27·7			
No. of cases/controls	256/254	247/254		235/254		273/254			188/199	147/200		193/199		263/199			
Crude	1	0·97	0·75, 1·24	0·92	0·72, 1·18	1·07	0·84, 1·36	0·70	1	0·78	0·58, 1·04	1·03	0·78, 1·36	1·40	1·07, 1·84	<0·01	
Adjusted*	1	0·85	0·63, 1·13	0·91	0·68, 1·21	1·00	0·74, 1·33	0·91	1	0·77	0·56, 1·07	0·99	0·73, 1·35	1·50	1·11, 2·03	<0·01	0·11
β-Sitostanol																	
Median intake (mg/d)	13·0	16·0		18·7		24·1			11·6	14·4		16·5		22·5			
No. of cases/controls	262/254	215/254		271/254		263/254			215/199	137/200		211/199		228/199			
Crude	1	0·82	0·64, 1·05	1·03	0·81, 1·32	1·00	0·79, 1·28	0·56	1	0·63	0·47, 0·85	0·98	0·75, 1·29	1·06	0·81, 1·39	0·21	
Adjusted*	1	0·84	0·63, 1·13	1·17	0·88, 1·56	1·27	0·94, 1·70	0·03	1	0·58	0·42, 0·80	0·93	0·69, 1·25	1·09	0·81, 1·46	0·16	0·42
Campestanol																	
Median intake (mg/d)	2·8	3·4		3·9		4·8			2·2	2·7		3·1		4·1			
No. of cases/controls	415/254	257/254		187/254		152/254			228/199	217/200		199/199		147/199			
Crude	1	0·62	0·49, 0·78	0·45	0·35, 0·58	0·37	0·28, 0·47	<0·01	1	0·95	0·73, 1·24	0·87	0·66, 1·15	0·65	0·49, 0·86	<0·01	
Adjusted*	1	0·81	0·62, 1·07	0·72	0·54, 0·97	0·63	0·46, 0·86	<0·01	1	0·97	0·72, 1·31	0·94	0·69, 1·27	0·78	0·57, 1·08	0·14	0·05

* OR was adjusted for age, marital status, residence, education, occupation, income level, BMI, smoking status, passive smoking, alcohol drinking, family history of cancer, occupational physical activity, household and leisure-time activities, dietary intake of dairy products, red and processed meat.

Table 5. OR of colorectal cancer according to quartiles (Q) of phytosterol intake by cancer site
(Odds ratios and 95 % confidence intervals)

	Colon cancer (n 1091)							Rectal cancer (n 711)						
	Q1	Q2		Q3		Q4		Q1	Q2		Q3		Q4	
	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}
Total phytosterols														
No. of cases/controls	386/453	287/453		227/454		191/453			309/453	160/453		125/454		117/453	
Crude	1	0.74	0.61, 0.91	0.59	0.48, 0.72	0.50	0.40, 0.62	<0.01	1	0.52	0.41, 0.65	0.40	0.32, 0.52	0.38	0.30, 0.49	<0.01
Adjusted*	1	0.77	0.62, 0.97	0.63	0.50, 0.80	0.57	0.45, 0.72	<0.01	1	0.55	0.43, 0.71	0.44	0.34, 0.58	0.45	0.34, 0.59	<0.01
β-Sitosterol														
No. of cases/controls	397/453	284/453		224/454		186/453			319/453	155/453		128/454		109/453	
Crude	1	0.72	0.59, 0.87	0.56	0.46, 0.69	0.47	0.38, 0.58	<0.01	1	0.49	0.39, 0.61	0.40	0.31, 0.51	0.34	0.27, 0.44	<0.01
Adjusted*	1	0.73	0.59, 0.92	0.62	0.49, 0.78	0.53	0.42, 0.67	<0.01	1	0.53	0.41, 0.68	0.45	0.35, 0.59	0.41	0.31, 0.54	<0.01
Campesterol														
No. of cases/controls	382/453	358/453		187/454		164/453			306/453	193/453		115/454		97/453	
Crude	1	0.94	0.77, 1.14	0.49	0.39, 0.61	0.43	0.34, 0.54	<0.01	1	0.63	0.51, 0.79	0.38	0.29, 0.48	0.32	0.24, 0.41	<0.01
Adjusted*	1	1.00	0.81, 1.24	0.55	0.43, 0.70	0.49	0.38, 0.62	<0.01	1	0.71	0.55, 0.90	0.43	0.33, 0.57	0.36	0.27, 0.47	<0.01
Stigmasterol														
No. of cases/controls	244/453	236/454		269/453		342/453			200/453	158/454		159/453		194/453	
Crude	1	0.97	0.77, 1.20	1.10	0.89, 1.37	1.40	1.14, 1.73	<0.01	1	0.79	0.62, 1.01	0.80	0.62, 1.02	0.97	0.77, 1.23	0.82
Adjusted*	1	0.88	0.69, 1.12	1.06	0.83, 1.34	1.41	1.12, 1.79	<0.01	1	0.76	0.58, 0.99	0.81	0.62, 1.06	1.07	0.83, 1.39	0.54
β-Sitostanol														
No. of cases/controls	269/453	215/454		291/453		316/453			208/453	137/454		191/453		175/453	
Crude	1	0.80	0.64, 1.00	1.08	0.88, 1.34	1.18	0.95, 1.45	0.02	1	0.66	0.51, 0.85	0.92	0.73, 1.16	0.84	0.66, 1.07	0.53
Adjusted*	1	0.77	0.60, 0.98	1.10	0.88, 1.39	1.35	1.07, 1.71	<0.01	1	0.69	0.53, 0.91	1.00	0.77, 1.29	1.05	0.80, 1.36	0.32
Campestanol														
No. of cases/controls	373/453	297/454		234/453		187/453			270/453	177/454		152/453		112/453	
Crude	1	0.79	0.65, 0.97	0.63	0.51, 0.77	0.50	0.40, 0.62	<0.01	1	0.65	0.52, 0.82	0.56	0.44, 0.71	0.42	0.32, 0.54	<0.01
Adjusted*	1	0.94	0.75, 1.18	0.85	0.67, 1.07	0.72	0.56, 0.92	0.01	1	0.73	0.57, 0.94	0.76	0.58, 1.00	0.59	0.44, 0.79	<0.01

* OR was adjusted for age, sex, marital status, residence, education, occupation, income level, BMI, smoking status, passive smoking, alcohol drinking, family history of cancer, occupational physical activity, household and leisure-time activities, dietary intake of dairy products, red and processed meat.

between the intake of total phytosterols, β-sitosterol and campesterol and colorectal cancer risk were more evident when using the hospital-derived controls than using the community-derived controls. Stigmasterol intake was positively associated with colorectal cancer risk when only using community-derived controls, whereas β-sitostanol intake was positively related to colorectal cancer risk when only using hospital-derived controls (Table 6). Sensitivity analysis by excluding participants who had dietary changes during the past 5 years (146 cases and 204 controls) did not materially change the results (data not shown). The energy-adjusted Pearson's correlation coefficient between intake of total phytosterols and total dietary fibre was 0·41 ($P < 0.01$) (data not shown).

Discussion

In this case–control study, a significant inverse association was found between the risk of colorectal cancer and the consumption of total phytosterols, β-sitosterol, campesterol and campestanol, whereas stigmasterol intake was related to an increased risk of colorectal cancer. Stratified analysis by sex showed that the positive association of stigmasterol intake with colorectal cancer risk was found only among women. Subgroup analysis by cancer site showed that stigmasterol and β-sitostanol intake was positively associated with colon cancer risk.

It was reported that the Seventh-day Adventists who consumed high levels of phytosterols in their diets had a decreased risk of developing colorectal cancer compared with the general population[19]. Moreover, a series of case–control studies performed in Uruguay revealed that dietary phytosterol intake exerts a potential protective effect on lung[14], breast[15], oesophageal[16] and stomach cancer[17]. These studies recruited 100–500 newly diagnosed patients with specific cancers. Total phytosterol intake was inversely associated with the risk of lung cancer for all cell types (OR 0·50; 95 % CI 0·31, 0·79, $P_{trend} < 0.01$), breast cancer (OR 0·37; 95 % CI 0·24, 0·57, $P_{trend} < 0.01$), oesophageal cancer (OR 0·27; 95 % CI 0·10, 0·70, $P_{trend} < 0.01$) and stomach cancer (OR 0·33; 95 % CI 0·17, 0·65, $P_{trend} = 0.01$). These findings are similar to our current results. However, in two other case–control studies from the USA, there was no significant association between total phytosterol intake and the risk of prostate[31] and ovarian[18] cancer. Instead, the consumption of stigmasterol was found to be positively associated with prostate cancer risk (OR 2·09; 95 % CI 1·19, 4·43, $P_{trend} = 0.03$) but inversely associated with ovarian cancer risk (OR 0·42; 95 % CI 0·20, 0·87, $P_{trend} < 0.05$). These two studies included eighty-three prostate cancer cases and 124 ovarian cancer cases. Moreover, in a case–cohort study conducted in the Netherlands, no reduction in colorectal cancer risk[20] was related to a high intake of any phytosterol.

Several possible explanations might account for these inconsistent results. First, dietary patterns vary between different countries. The amount of phytosterol consumption might be different due to differences in phytosterol content in food. In our study, the mean intake of total phytosterols among the controls was 225·9 mg/d, which was derived from plant oils, cereal products, vegetables and fruits, legumes and nuts. In the

Uruguayan case–control study[14], the mean consumption of total phytosterols among controls was 144 mg/d and the main food sources were maize, legumes, bananas and apples. The average American diet[9] contained 154 mg of phytosterols for adults consuming 8368 kJ/d (2000 kcal/d). The Netherlands study mentioned above reported that the daily phytosterol intake was 307 mg for men and 263 mg for women[20]. The total consumption of β-sitosterol and stigmasterol among the non-vegetarians in American general population was 77·9 mg/d[19]. Second, factors such as the type of phytosterol (plant sterol or plant stanol), the phytosterol sources and the cooking methods used may affect the bioavailability and bioactivity of phytosterols[9]. A previous investigation using animal models found that intestinal absorption of phytosterols was selective: campesterol was absorbed better than β-sitosterol and stigmasterol was only minimally absorbed[32]. In addition, refined grain products contain fewer phytosterols than their whole grain counterparts[10] and boiling may variably affect the phytosterol content of vegetables[33].

The potential mechanisms of the protective effect of most of the phytosterols against colorectal cancer include inhibition of the cell cycle progression, induction of cellular apoptosis and the reduction of cellular oxidative stress. In a study of the effects of β-sitosterol on the growth of HCT116 human colon cancer cells, Choi et al.[12] found a dose-dependent growth inhibition coupled with an increase in a sub-G1 cell population. β-Sitosterol induced cell cycle arrest by regulating the expression of apoptosis-related proteins. Moreover, Baskar et al.[13] reported that β-sitosterol inhibited the depletion of antioxidant enzymes and restored the levels of nonenzymatic antioxidants in a 1,2-dimethylhydrazine-induced colon carcinogenesis rat model. This suggests that β-sitosterol possesses antioxidant properties that can protect cells from damage by reactive oxygen species. In addition, previous prospective studies reported that a high level of serum total cholesterol[34] and a high dietary cholesterol intake[35] was associated with an increased risk of colorectal cancer. As phytosterols are structurally similar to cholesterol, phytosterols can compete with cholesterol absorption in the intestines, leading to lower serum cholesterol levels[36] and inhibiting the proliferation of cancer cells[37].

The result of the positive association between stigmasterol intake and colorectal cancer risk was contrary to our hypothesis. Probably, it was only a chance finding. Although one case–control study from the USA[31] reported positive association between stigmasterol intake and prostate cancer risk and one case–cohort study from the Netherlands[20] reported positive association between stigmasterol intake and colon cancer among men, it was not possible to draw any firm conclusions at this time. More studies, especially with prospective study design, are needed to clarify this association.

Stratified analysis by sex showed that the intake of stigmasterol was positively related to the risk of colorectal cancer only among women. Similarly, one case–cohort study conducted in the Netherlands[20] also observed a positive association between the consumption of stigmasterol and proximal colon cancer among women (OR 2·52; 95 % CI 1·34, 4·75). This sex difference may be due to dietary complexities. It was reported that typical dietary patterns differed between men

Table 6. OR of colorectal cancer according to quartiles of phytosterol intake in two control groups
(Odds ratios and 95 % confidence intervals)

	Hospital-derived controls (n 924)								Community-derived controls (n 889)							
	Q1	Q2		Q3		Q4			Q1	Q2		Q3		Q4		
	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}
Total phytosterols																
No. of cases/controls	695/182	447/237		352/272		308/233			695/271	447/216		352/182		308/220		
Crude	1	0·49	0·39, 0·62	0·34	0·27, 0·43	0·35	0·27, 0·44	<0·01	1	0·81	0·65, 1·00	0·75	0·60, 0·95	0·55	0·44, 0·68	<0·01
Adjusted*	1	0·49	0·39, 0·63	0·35	0·27, 0·45	0·36	0·27, 0·46	<0·01	1	0·89	0·66, 1·20	0·75	0·55, 1·03	0·65	0·48, 0·89	<0·01
β-Sitosterol																
No. of cases/controls	716/187	439/235		352/276		295/226			716/266	439/218		352/178		295/227		
Crude	1	0·49	0·39, 0·61	0·33	0·27, 0·42	0·34	0·27, 0·43	<0·01	1	0·75	0·60, 0·93	0·74	0·58, 0·92	0·48	0·39, 0·60	<0·01
Adjusted*	1	0·48	0·38, 0·62	0·34	0·27, 0·43	0·35	0·27, 0·45	<0·01	1	0·83	0·62, 1·12	0·86	0·63, 1·17	0·55	0·40, 0·75	<0·01
Campesterol																
No. of cases/controls	688/197	551/219		302/213		261/295			688/256	551/234		302/241		261/158		
Crude	1	0·72	0·58, 0·90	0·41	0·32, 0·51	0·25	0·20, 0·32	<0·01	1	0·88	0·71, 1·08	0·47	0·37, 0·58	0·62	0·48, 0·79	<0·01
Adjusted*	1	0·77	0·60, 0·98	0·44	0·34, 0·57	0·27	0·21, 0·34	<0·01	1	0·90	0·67, 1·19	0·47	0·34, 0·65	0·74	0·53, 1·03	<0·01
Stigmasterol																
No. of cases/controls	444/193	394/233		428/249		536/249			444/260	394/221		428/204		536/204		
Crude	1	0·74	0·58, 0·93	0·75	0·59, 0·94	0·94	0·75, 1·17	0·77	1	1·04	0·83, 1·31	1·23	0·98, 1·54	1·54	1·23, 1·92	<0·01
Adjusted*	1	0·73	0·57, 0·95	0·74	0·58, 0·95	1·00	0·78, 1·28	0·80	1	1·02	0·75, 1·39	1·26	0·92, 1·72	1·72	1·26, 2·35	<0·01
β-Sitostanol																
No. of cases/controls	477/239	352/255		482/262		491/168			477/214	352/199		482/191		491/285		
Crude	1	0·69	0·55, 0·87	0·92	0·74, 1·14	1·46	1·16, 1·85	<0·01	1	0·79	0·63, 1·01	1·13	0·90, 1·43	0·77	0·62, 0·96	0·15
Adjusted*	1	0·70	0·54, 0·89	0·97	0·76, 1·23	1·57	1·21, 2·02	<0·01	1	0·84	0·60, 1·16	1·16	0·84, 1·59	0·93	0·69, 1·26	0·91
Campestanol																
No. of cases/controls	643/262	474/254		386/233		299/175			643/191	474/200		386/220		299/278		
Crude	1	0·76	0·62, 0·94	0·68	0·54, 0·84	0·70	0·55, 0·88	<0·01	1	0·70	0·56, 0·89	0·52	0·41, 0·66	0·32	0·25, 0·40	<0·01
Adjusted*	1	0·80	0·63, 1·01	0·74	0·58, 0·94	0·72	0·55, 0·94	0·01	1	1·03	0·75, 1·40	0·80	0·58, 1·11	0·64	0·47, 0·88	<0·01

* OR was adjusted for age, sex, marital status, residence, education, occupation, income level, BMI, smoking status, passive smoking, alcohol drinking, family history of cancer, occupational physical activity, household and leisure-time activities, dietary intake of dairy products, red and processed meat.

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and women[38], suggesting that different combinations of food groups or nutrients in men's and women's diets may have distinct effects on the carcinogenesis of colorectal cancer. As shown in our study, the amount of phytosterols intake among men was higher than that among women, which may partly explain the stronger inverse association found in men. Moreover, tumour carcinogenesis may be regulated by sex hormones. An animal study indicated that high intake of plant sterols accelerated intestinal tumourigenesis in female $(Apc)^{Min}$ mice[39], and reduced the concentration of free cholesterol in intestinal mucosa only in male mice. A significant positive correlation was found between tumour number and the level of β-oestrogen receptors in female mice. One study in Korea reported that the ratio of the α- and β-oestrogen receptors was important with regard to colorectal cancer risk[40]. However, to date, the mechanism underlying this sex difference has not been clarified. Therefore, whether the association between phytosterols and colorectal cancer risk is modified by sex still needs further investigation.

Subgroup analysis by cancer site presented a positive association between intake of stigmasterol, β-sitostanol and the risk of colon cancer. Similar to these results, one case–cohort study in the Netherlands[20] observed that higher consumption of stigmasterol was related to an increased risk of colon cancer in men (OR 1·84; 95 % CI 1·14, 2·96), and an increased risk of proximal colon cancer in women (OR 2·52; 95 % CI 1·34, 4·75). Furthermore, animal study[41] found that feeding the rats with a mixture of phytosterols as the human diet had no protective effect on the colon carcinogenesis. The probable reason of which was the phytosterols supplement had a negative effect on bacterial activity[41]. Nevertheless, this issue needs to be justified with further studies.

Both hospital-derived and community-derived controls were used in this study to strengthen the association between dietary phytosterols intake and colorectal cancer risk. The results showed that the inverse associations between the intake of total phytosterols, β-sitosterol and campesterol, and colorectal cancer risk were more evident when using the hospital-derived controls than using the community-derived controls. One possible reason might be that the control subjects admitted to the hospitals were much younger than the community residents. The average age was about 51·0 years for hospital-derived controls and 61·5 years for community-derived controls, respectively. It is well-known that the incidence of cancer increases with age[42]. Therefore, it was likely that the protective effect of phytosterols intake was stronger when using the younger hospital-derived controls than by using older community-derived controls. The positive association of stigmasterol intake with colorectal cancer risk was observed when using only community-derived controls, and the positive association of β-sitostanol intake with colorectal cancer risk was found when using only hospital-derived controls. This might be a chance finding. More studies are needed to clarify this association.

It was reported that a higher dietary fibre intake was associated with a reduced risk of colorectal cancer[43]. So, dietary fibre may be a potential confounder when examining the association between phytosterols intake and colorectal cancer risk. The intake of dietary fibre and total phytosterols was significantly correlated in the present study, with the correlation coefficient of 0·41. Therefore, to avoid the over-adjustment, dietary fibre was not included in the multivariable model. Moreover, it was reported that about 60 % of dietary fibre was derived from vegetables and fruits in Chinese population[44]. However, vegetables and fruits only contributed 13·5 % to the total phytosterols intake[10]. About 40 % of the total phytosterols intake in Chinese people was from edible oil[10], which was free from dietary fibre. Therefore, the inverse association between phytosterols intake and colorectal cancer risk was probably not confounded by dietary fibre.

The present study has the following strengths. First, a validated FFQ was used to assess the frequency and quantity of phytosterol intake. Second, we adjusted several potential confounders, including dietary and non-dietary factors, in the analysis. In addition, the sample size is larger than in previous relevant case–control studies: we therefore have enough power to detect small associations with the risk of colorectal cancer. Furthermore, this is the first study to examine the relationship between phytosterol intake and colorectal cancer risk in the Chinese population, which might help to produce targeted dietary guidance for this group.

The present study has several potential limitations. First, selection bias is difficult to rule out in hospital-based case–control studies. Although the colorectal cancer patients were recruited from only one hospital, Sun Yat-sen University Cancer Center, it is the biggest cancer centre in Southern China. The colorectal patients at this centre and those from other two big hospitals in Guangdong or in mainland China therefore shared similar clinical characteristics. Moreover, the high participation rate (90 % for cases and 87 % for hospital-derived controls) also helped to reduce selection bias in our results. Second, as in all case–control studies, recall bias is inevitable. To minimise this bias, we included only newly diagnosed cases and interviewed them as soon as possible after diagnosis. The average time interval between the diagnosis of colorectal cancer and study interview was 11·0 d for the case subjects. In addition, food photographs with usual portion sizes were also provided to help the participants to estimate their intake more accurately. Third, random measurement error of diet is also of concern in the estimation of usual intake, since this misclassification bias is likely to be nondifferential, leading the OR to the null. This measurement error is therefore of less importance and our results may tend to be conservative. Fourth, although various dietary and non-dietary factors were adjusted in our multivariable analysis, residual confounding factors may still remain. Fifth, there is a concern that colorectal cancer cases might change their dietary habits due to pre-diagnosis symptoms and lead to the lower nutrients and food intake. However, sensitivity analysis by excluding study subjects with dietary changes showed no substantial change. Therefore, the pre-diagnosis symptoms of colorectal cancer, even if they might have, should not be a serious problem.

In conclusion, this study shows that dietary intakes of total phytosterols, β-sitosterol, campesterol and campestanol are inversely associated with colorectal cancer risk in a Chinese population. The positive association between stigmasterol

intake and colorectal cancer risk observed in the present study needs to be further justified.

Acknowledgements

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