Nutrition Journal

RESEARCH

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Consumption of different types of meat and the risk of chronic limb-threatening ischemia: the Singapore Chinese Health Study

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Abstract

Background Although red meat consumption has been associated with risk of atherosclerotic coronary artery disease and stroke, no prospective study has examined this with the risk of chronic limb-threatening ischemia (CLTI).

Methods In a prospective study of 63,257 Chinese in Singapore, who were aged 45–74 years old at recruitment, diet was assessed via a validated semi-quantitative food frequency questionnaire. Incident CLTI cases were ascertained via linkage with nationwide hospital records for lower extremity amputation or angioplasty for peripheral arterial disease. Multivariable Cox models were used to examine associations between quartiles of meat intake and CLTI risk.

Results After a mean follow-up of 18.8 years, there were 1069 cases of CLTI. Higher intake of red meat intake was associated with increased risk of CLTI in a stepwise manner. Comparing extreme quartiles of red meat intake, the hazard ratio (HR) for the association with CLTI risk was 1.24 [95% confidence interval (CI) = 1.03–1.49; *P*-trend = 0.02]. In stratified analysis, red meat intake had a stronger association with CLTI risk among those without diabetes [HR (95% CI) comparing extreme quartiles = 1.41 (1.10–1.80); *P*-trend = 0.03] than among those with diabetes at baseline [HR (95% CI) comparing extreme quartiles = 1.04 (0.79–1.38); *P*-trend = 0.05] (P-interaction = 0.03). Otherwise, the associations were not different by sex, BMI, smoking status, hypertension, alcohol consumption, or history of cardiovascular diseases. Using a theoretical model in substitution analysis that substituted three servings per week of red meat with poultry or fish/shellfish, the relative risk of CLTI was reduced by 13–14%.

Conclusions Consumption of red meat was associated with higher CLTI risk in this Asian cohort. Substituting red meat with poultry or fish/shellfish may reduce this risk.

Keywords Diet, Peripheral arterial disease, Chronic limb-threatening ischemia, Red meat, Cohort study

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Introduction

Chronic limb-threatening ischemia (CLTI) is the most severe manifestation of peripheral arterial disease (PAD), which is an atherosclerotic disease that affects the arteries in the lower limbs. In patients with CLTI, the arterial stenosis is sufficiently severe to cause ischemic rest pain, non-healing ulcers or gangrene, and often results in amputation, impaired quality of life, and a high risk of mortality [1].

The incidence of CLTI has been steadily increasing globally in recent years, and this has partially been attributed to an increase in the prevalence of its risk factors [1, 2]. In particular, the rising prevalence of diabetes is a cause for concern, [3, 4] as diabetes is a strong risk factor for PAD, and PAD patients with diabetes are also at a much higher risk of amputations and subsequent mortality than PAD patients who do not have diabetes [5, 6]. Besides diabetes, other established vascular risk factors, such as cigarette smoking, hypertension, hyperlipidemia and obesity, have also been associated with increased risk of PAD and CLTI [7].

Epidemiologic evidence has established dietary risk factors in association with other atherosclerotic diseases, such as coronary artery disease and stroke [8, 9]. Hence, other than vascular risk factors, it is possible that diet also plays an important role in the pathogenesis of PAD and CLTI. Specifically, previous studies have observed an association between higher red meat intake and an increased risk of coronary artery disease and stroke [10, 11]. Therefore, it is not surprising that similar associations have been reported for PAD. For example, the Edinburgh Artery Study in 1993 observed that a higher intake of meat and meat products was significantly associated with low mean ankle-brachial pressure index in both men and women [12]. More recently in 2017, the Atherosclerosis Risk in Communities (ARIC) Study cohort reported that participants with higher meat intake were at higher risk of PAD after a mean follow-up duration of 19.9 years [13]. Cohort studies in Sweden and Denmark have further examined the effects of different types of meat on PAD risk and concluded that intake of red meat was associated with the risk of PAD [14, 15].

However, in the aforementioned studies, cases of PAD ranged from asymptomatic stage defined by an ankle-brachial index of less than 0.90 to critical stages that necessitated lower extremity amputations (LEA); thus, were not uniform in the severity of disease presentation. Furthermore, all these studies were conducted in Western populations, and to our best knowledge, no study on diet and PAD has been conducted in an Asian population, which may differ in meat intake from Western populations in terms of the absolute amount of intake and the type of red meat (pork versus beef) consumed [16]. PAD remains an under-diagnosed and under-investigated disease in Asian populations, and the disease pattern and underlying risk factors may also be different from those in Western populations [2, 17].

In the present study, we investigated associations between the consumption of red meat, poultry, and fish/shellfish, and the risk of developing CLTI in a population-based cohort of Chinese living in Singapore. To assess whether a change in meat consumption could potentially reduce any increased risk, we also examined the effects of substituting red meat with poultry or fish/shellfish on CLTI risk.

Research design and methods

Study population

This study used data from the Singapore Chinese Health Study, a large prospective population-based cohort established in 1993–1998 by the recruitment of 27,959 men and 35,298 women aged 45–74 years. They were drawn from a pool of permanent residents and citizens who resided in government purpose-built housing estates, where 86% of the Singapore population resided at the time of recruitment [18]. The study was approved by the institutional review board at the National University of Singapore, and informed consent was obtained from all participants.

Assessment of diet and other covariates

All participants were interviewed at their homes by trained interviewers using structured questionnaires. We collected information on age, weight, height, dialect group, level of education, weekly physical activity, smoking status, and alcohol intake. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters. Self-reported information on physician-diagnosed chronic diseases, such as diabetes and hypertension, was also obtained.

Diet over the past year was assessed via a semi-quantitative food frequency questionnaire (FFQ) that was specifically developed for this study population. The FFQ contained 165 food items common in the Singapore Chinese diet, identified from 400 person-days of food intake in a pilot study [18]. In brief, 200 Chinese (50 each of Cantonese and Hokkien men and women) reported their food intake on one weekday and one weekend for each person. In Chinese dishes commonly consumed in this cohort, different types of meat were not eaten in isolation. Instead, a small quantity of meat was often prepared together with vegetables, staples such as rice or noodles, and condiments, and subsequently served as one mixed dish. Hence, after identifying common food items consumed by this population, we purchased multiple samples of the same dish, directly weighed the ingredient components, and developed recipes to quantify the amount of meat, vegetables, staples and seasonings used in each dish. Due to this nature of Chinese cuisine, we reported our findings in the form of grams per day instead of servings, as every dish would have its own serving size of meat.

For each food item, participants referred to an actual plate/bowl and photographs of the food item on the same plate/bowl to select from eight intake frequencies (from “never or hardly ever” to “two or more times a day”) and three portion sizes (small, medium, or large). Meat consumption was assessed in 33 FFQ questions: eight on red meat, seven on poultry, six on fish/shellfish, and 12 on preserved/processed meat, and then converted to grams using the Singapore Food Composition Database that was developed specifically for this cohort [18].

Validation of the FFQ was performed in a subset of 810 participants using a re-administration of the FFQ and two 24-hour recalls. Similar distributions were observed using both methods, with the mean value of most pairs for energy and nutrients being within 10% of each other, and a correlation coefficient ranging from 0.24 to 0.79, comparable to validation studies performed in other cohorts [19]. Correlation coefficients were expressed as a range as they included eleven nutrients calculated via three methods (absolute values, calorie-adjusted values, and proportion of nutrient density) for two different dialect groups (Hokkien and Cantonese) and two sexes (men and women) [18]. Although correlation coefficients were not computed specifically for each type of meat, the correlation coefficients for protein and fat intake, which were derived mainly from meat in this population, ranged from 0.36 to 0.61 for protein and 0.34–0.74 for fat [18].

Ascertainment of CLTI and follow-up

Incident CLTI cases, defined as participants who underwent LEA or angioplasty for PAD after enrolment into the cohort, were determined via record linkage of the cohort database with the Singaporean MediClaim System, a government-established, nation-wide database that has captured all inpatient discharge information from all private and government restructured hospitals in Singapore since 1990. Cases were identified by records of the appropriate surgical procedural codes, namely major, minor, or digital amputation in the lower limb, or angioplasty. Due to the presentation of PAD in the local population, which was predominantly below-knee disease with long calcified segments, these are the main surgical procedures performed for PAD in Singapore [20]. Only the first instance of surgical procedure was recorded for each case. We then excluded 59 LEAs performed for non-vascular causes, which were identified using International Classification of Disease Version 9 (ICD-9) and Version 10 (ICD-10) codes for trauma (ICD-9 800–999; ICD-10 S00-Y99), peripheral neuropathy (ICD-9 355, 356, 357; ICD-10 G57, G60-G65), cancers of the bone, cartilage, and nerves (ICD-9 170.6-170.9, 171.3, 171.6-171.9; ICD-10 C40.2-C40.9, C47.2, C47.5-C47.9), necrotizing fasciitis (ICD-9 728.86; ICD-10 M72.6), and osteomyelitis or osteonecrosis (ICD-9 730; ICD-10 M86-M87).

Information on date of death was determined via record linkage of the cohort database with the nationwide Registry of Births and Deaths database. As of 31 December 2017, which was the censored date for record linkages with the MediClaim System and death databases, only 41 participants (0.06%) in this cohort were lost to follow-up due to reasons such as emigration out of Singapore. As such, data capture was virtually complete.

Statistical analysis

Of the 63,257 participants enrolled into the study, 123 of them had developed CLTI before recruitment and were hence excluded from the study. We further excluded 472 men who reported < 700 or > 3700 kcal/day of dietary intake and 584 women who reported < 600 or > 3000 kcal/day of dietary intake (Fig. 1). For the remaining 62,078 participants, person-years were calculated from the date of recruitment to the date of CLTI procedure, date of death, or 31 December 2017, whichever came first. ANOVA or chi-squared test was used to compare the difference between categorical or continuous variables amongst the four quartiles of red meat intake.

Multivariable-adjusted Cox models were used to examine associations between consumptions of red meat, poultry, and fish/shellfish with CLTI risk. Daily intake of each type of meat, fruits, and vegetables was adjusted for total energy intake using the residual method to remove confounding arising from differences in total energy intake [21]. Participants in the lowest quartile of intake for each type of meat was used as the reference group. Model 1 adjusted for five demographics variables, namely sex, year of study enrolment (1993–1995, 1996–1998), dialect group (Hokkien, Cantonese), educational level (no formal education, primary school, secondary school or higher), and weekly physical activity (< 0.5 h/week, 0.5-< 4 h/week, ≥ 4 h/week), as well as eight known risk factors for atherosclerosis, namely age at recruitment (year), BMI (< 18.5 kg/m^2, 18.5 kg/m^2-22.9 kg/m^2, 23 kg/m^2-27.4 kg/m^2, > 27.5 kg/m^2), smoking (never, former, current), alcohol consumption (never/monthly, weekly/daily), history of hypertension (yes, no), history of diabetes (yes, no), history of coronary artery disease (yes, no), history of stroke (yes, no), and the dietary variable total energy intake (kcal/day). Physical activity was defined as any moderate activity, vigorous activity, or strenuous sports activity lasting at least 30 min per week. Model 2 further adjusted for daily intake of fruits and vegetables as quartiles. Finally, in addition, Model 3 also mutually adjusted for red meat, poultry and fish/shellfish as quartiles. To study linear trends, we first allocated the median

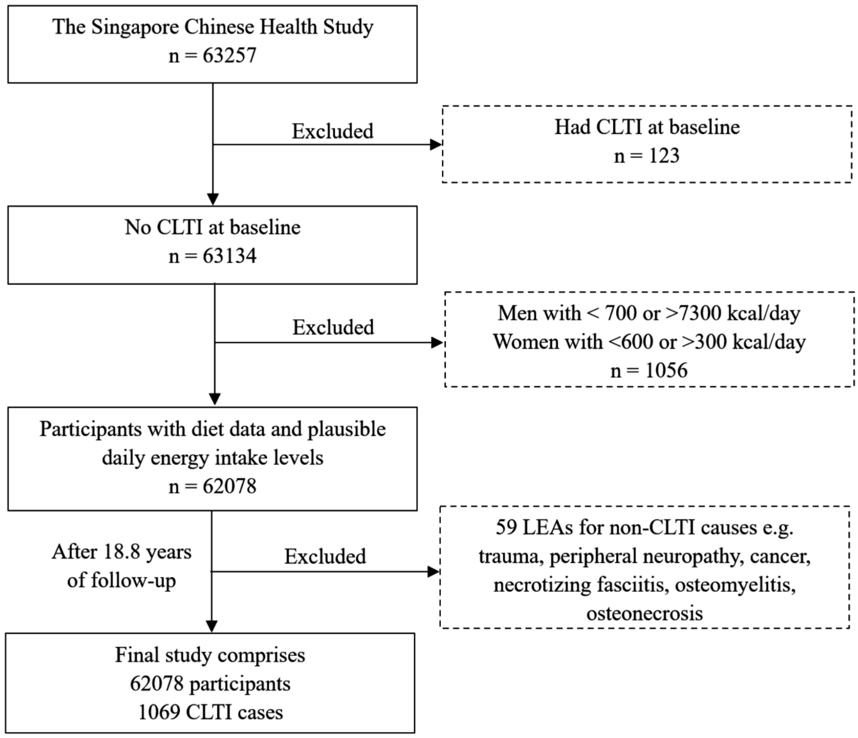

Fig. 1 Flow diagram of the Singapore Chinese Health Study and the exclusion criteria of this study

intake value of each quartile to all the participants in the respective quartile. Then we estimated the linear trend between stepwise increase in intake and the risk of CLTI by using the median values of the quartiles as a continuous variable in the Cox proportional hazards model. To study interactions, the product term between red meat intake and each vascular risk factor (sex, BMI, smoking status, alcohol consumption, diabetes status, and hypertension status) was included as interaction terms in the Cox models.

The effect of substituting three servings (150 g) of red meat per week with poultry or fish/shellfish was modelled using the method modified from one previously described by Kulldorff et al., [22] which derived the hazard ratio of substitution by simultaneously including intake of both types of meat in a multivariable Cox regression model, calculating the exponential of the difference between the two coefficients, and then using the covariance between them to derive the corresponding 95% CI. One serving of meat was defined as 50 g as this is the serving size most commonly used by Chinese populations [23].

Substitution analysis was performed using Stata/SE (version 14.2; Stata Corporation, College Station, TX, USA). All other analyses were performed using SAS (version 3.81; SAS Institute, Inc., Cary, NC, USA). All p-values presented are two-sided, and p-value < 0.05 was considered statistically significant.

Results

After a mean (±SD) follow-up duration of 18.8 (±6.2) years, there were 1,069 incident cases of CLTI. Median intake of meat in this cohort was a total of 90.8 g/day (interquartile range: 55.4–137.0 g/day), and on average, 27.3% was red meat (pork, beef, lamb, and mutton), 17.0% was poultry (chicken and duck), and 55.7% was fish/shellfish. The majority of red meat consumed was in the form of fresh red meat (92.8%), while preserved red meat and organ red meat accounted for 5.5% and 1.7% of red meat consumption, respectively. Pairwise Pearson correlation coefficients for meat intakes were 0.50 for red meat and poultry, 0.42 for red meat and fish/shellfish, and 0.33 for poultry and fish/shellfish.

The baseline characteristics of participants in the first and fourth quartiles of red meat intakes are presented in Table 1. Compared to participants in the lowest quartile of intake, participants in the highest quartile of red meat consumption were slightly younger at recruitment (55.8 years old versus 56.6 years old), less likely to engage in physical activity (12.4% versus 16.0% for ≥4 h/week), and more likely to be current smokers (23.4% versus 18.3%). Interestingly, those in the highest quartile of intake were more likely to have diabetes (9.4% versus 7.4%), but were less likely to have hypertension (22.3% versus 24.1%) and a history of coronary artery disease (3.6% versus 4.3%) or stroke (1.2% versus 1.8%) (p-values < 0.01). They also

Table 1 Participant characteristics according to quartiles of red meat intake, the Singapore Chinese Health Study

	Q1	Q2	Q3	Q4
Number of subjects	15,656	15,696	15,351	15,375
Number of CLTI cases	243 (1.6%)	259 (1.7%)	276 (1.8%)	291 (1.9%)
Age in years, mean (SD)	56.6 (7.9)	57.0 (8.1)	56.6 (8.1)	55.8 (7.9)
BMI in kg/m^2, mean (SD)	23.0 (3.3)	23.1 (3.2)	23.2 (3.3)	23.2 (3.3)
Sex				
Men	7741 (49.4%)	6085 (38.8%)	6044 (39.4%)	7538 (49.0%)
Women	7915 (50.6%)	9611 (61.2%)	9307 (60.6%)	7837 (51.0%)
Dialect				
Hokkien	7964 (50.9%)	7266 (42.3%)	6723 (43.8%)	6874 (44.7%)
Cantonese	7692 (49.1%)	8430 (53.7%)	8628 (56.2%)	8501 (55.3%)
Level of education				
No formal education	3873 (24.7%)	4625 (29.5%)	4513 (29.4%)	3884 (25.3%)
Primary school	6991 (44.7%)	6849 (43.6%)	6797 (44.3%)	6950 (45.2%)
Secondary or higher	4792 (30.6%)	4222 (26.9%)	4041 (26.3%)	4541 (29.5%)
Weekly physical activity				
< 0.5 h/week	9625 (61.5%)	10,588 (67.5%)	10,792 (70.3%)	10,629 (69.1%)
0.5 to < 4 h/week	3529 (22.5%)	3268 (20.8%)	2933 (19.1%)	2846 (18.5%)
≥ 4 h/week	2502 (16.0%)	1840 (11.7%)	1626 (10.6%)	1900 (12.4%)
Smoking				
Never	10,793 (68.9%)	11,433 (72.8%)	10,928 (71.2%)	10,026 (65.2%)
Former	2002 (12.8%)	1582 (10.1%)	1512 (9.8%)	1753 (11.4%)
Current	2861 (18.3%)	2681 (17.1%)	2911 (19.0%)	3596 (23.4%)
Alcohol drinking				
Never/monthly	13,634 (87.1%)	14,111 (89.9%)	13,833 (90.1%)	13,365 (86.9%)
Weekly/daily	2022 (12.9%)	1585 (10.1%)	1518 (9.9%)	2010 (13.1%)
Medical History				
Diabetes	1165 (7.4%)	1420 (9.1%)	1455 (9.5%)	1446 (9.4%)
Hypertension	3768 (24.1%)	3961 (25.2%)	3593 (23.4%)	3428 (22.3%)
Coronary artery disease	674 (4.3%)	694 (4.4%)	618 (4.0%)	554 (3.6%)
Stroke	274 (1.8%)	231 (1.5%)	225 (1.5%)	188 (1.2%)
Mean daily dietary intake				
Total energy, kcal/day (SD)	1763.2 (480.7)	1417.4 (452.8)	1381.5 (472.5)	1620.4 (566.2)
Red meat, g/day (SD)	16.0 (11.5)	20.4 (12.4)	28.9 (13.3)	55.4 (25.6)
Poultry, g/day (SD)	17.8 (18.2)	17.3 (16.0)	19.5 (16.7)	26.5 (21.1)
Fish/shellfish, g/day (SD)	54.6 (33.6)	49.9 (28.0)	52.2 (27.4)	63.0 (31.6)
Vegetables, g/day (SD)	126.2 (74.7)	102.7 (55.1)	99.4 (52.8)	111.7 (57.3)
Fruits, g/day (SD)	259.5 (203.6)	194.0 (148.2)	173.5 (138.7)	177.7 (141.3)

ANOVA or chi-squared test was used to compare the difference between categorical or continuous variables amongst the four quartiles of red meat intake; $p < 0.05$ for all

ate more poultry and fish/shellfish, but less fruits and vegetables.

Table 2 shows the associations between different types of meat intake and the risk of developing CLTI. In multivariable models that adjusted for all potential confounders, including vascular risk factors plus intake of fruits, vegetables, and other types of meat (Model 3), the consumption of red meat was positively associated with CLTI risk; the HR (95% CI) comparing extreme quartiles was 1.24 (1.03–1.49; p for trend = 0.02). In contrast, there was no significant association between intake of poultry or fish/shellfish intake and CLTI risk. We also repeated the analysis as servings per week and the observations remained the same, whereby those who consumed 7+ servings of red meat a week (i.e. at least once a day) had a higher risk of CLTI than those who consumed 2 or less servings a week, with a HR (95% CI) of 1.41 (1.14–1.75). Conversely, increased servings of poultry or fish/shellfish were not associated with CLTI risk (Supplementary Table 1).

In stratified analysis, red meat intake had a stronger association with CLTI risk among those without diabetes at baseline [HR (95% CI) comparing extreme quartiles = 1.41 (1.10–1.80); p for trend = 0.03] than among those with diabetes at baseline [HR (95% CI) comparing extreme quartiles = 1.04 (0.79–1.38); p for trend = 0.05]

Table 2 Association between quartiles of meat intake and the risk of developing chronic limb-threatening ischemia

Quartiles	Mean (g/day)	Person-years	Cases	Model 1 HR (95% CI)†	Model 2 HR (95% CI)‡	Model 3 HR (95% CI)§
Red Meat						
Q1	16.0	297,018	243	1.00	1.00	1.00
Q2	20.4	296,271	259	1.04 (0.87–1.24)	1.05 (0.88–1.26)	1.06 (0.88–1.27)
Q3	28.9	288,308	276	1.15 (0.96–1.37)	1.17 (0.97–1.40)	1.20 (0.99–1.45)
Q4	55.4	288,388	291	1.16 (0.98–1.38)	1.19 (1.01–1.42)	1.24 (1.03–1.49)
P for trend*				0.09	0.05	0.02
Poultry						
Q1	8.6	289,136	275	1.00	1.00	1.00
Q2	11.6	290,555	293	1.07 (0.90–1.27)	1.07 (0.90–1.27)	1.04 (0.87–1.24)
Q3	18.7	290,627	264	1.05 (0.88–1.25)	1.05 (0.88–1.26)	1.00 (0.83–1.20)
Q4	42.3	299,667	237	0.91 (0.77–1.09)	0.92 (0.77–1.10)	0.87 (0.72–1.04)
P for trend*				0.13	0.17	0.05
Fish/Shellfish						
Q1	28.2	286,684	260	1.00	1.00	1.00
Q2	42.3	289,878	275	1.07 (0.90–1.27)	1.08 (0.91–1.29)	1.06 (0.89–1.26)
Q3	57.8	295,566	263	0.97 (0.82–1.16)	1.00 (0.84–1.19)	0.97 (0.81–1.16)
Q4	91.1	297,858	271	0.93 (0.78–1.10)	0.96 (0.81–1.15)	0.93 (0.78–1.11)
P for trend*				0.21	0.41	0.23

*Linear trend was tested using the median values of quartiles as a continuous variable in Cox proportional hazards models

†Model 1: Hazard ratio (HR) adjusted for age at recruitment (year), year of study enrolment (1993–1995, 1996–1998), dialect group (Hokkien, Cantonese), educational level (no formal education, primary school, secondary school or higher), weekly physical activity (<0.5 h/week, 0.5-<4 h/week, ≥4 h/week), sex (men, women), BMI (<18.5 kg/m^2, 18.5 kg/m^2-22.9 kg/m^2, 23 kg/m^2-27.4 kg/m^2, >27.5 kg/m^2), smoking (never, former, current), alcohol consumption (never/monthly, weekly/daily), history of hypertension (yes, no), history of diabetes (yes, no), history of CAD (yes, no), history of stroke (yes, no), and total energy intake (kcal/day)

‡Model 2: Hazard ratio (HR) adjusted for Model 1 plus intake of fruits and vegetables in quartiles

§Model 3: Hazard ratio (HR) adjusted for Model 2 plus intake of the other two types of meat in quartiles

(*p* for interaction = 0.03). Otherwise, the associations did not differ by sex, BMI, smoking status, hypertension, or alcohol consumption (Table 3).

We also conducted sensitivity analyses that included 4-year lag time, excluded those with cardiovascular disease at baseline, and excluded those with cancer at baseline, and the results remained materially the same (Supplementary Table 2).

We further examined for changes in relative risk when one serving of a specific type of meat was substituted by a different type of meat using a nutritional substitution model in Fig. 2. For three 50-gram servings of red meat a week, replacing them with three servings of poultry was associated with 14% lower relative CLTI risk (HR, 0.86; 95% CI, 0.76–0.97), and replacing them with three servings of fish/shellfish was associated with 13% lower risk (HR, 0.87; 95% CI, 0.79–0.96).

Discussion

In this large prospective population-based cohort of middle-aged and elderly Chinese persons, we found that consumption of red meat was associated with higher CLTI risk, and this association seemed to be stronger among those without diabetes at baseline than among those with diabetes. Using a theoretical model to substitute red meat with poultry or fish/shellfish showed a statistically significant reduction in CLTI risk.

Our study concurs with results reported by the Danish Diet, Cancer and Health Cohort, which followed 54,597 participants from Denmark across a median of 13.6 years [14]. This Dutch study reported that substituting either unprocessed or processed red meat with fish reduced relative CLTI risk by 8% [14]. Our study also concurs with results from the ARIC study, which followed 14,082 participants across a mean of 19.9 years, and reported that participants who ate more red meat were at higher risk of PAD, while those who ate more poultry and fish/seafood did not have increased risk [13]. In contrast, the Swedish Mammography Cohort and Cohort of Swedish Men, which followed 82,295 participants across a median of 22 years, reported that increased PAD risk was associated with intake of processed red meat, but not with intake of unprocessed red meat [15]. This latter discrepancy may be explained by differences in the type of red meat between cohorts and in the outcome measures between studies. In the present study, the cohort comprised Chinese participants whose red meat consumption was 97% pork, [16] whereas a European diet might contain much more beef or veal [24]. Hence, in the present study, we are only able to draw extrapolations to pork consumption, and would be less able to comment on associations between beef/veal and CLTI risk.

To the best of our knowledge, this is the first prospective Asian cohort that has shown a positive association

Table 3 Association between red meat intake and CLTI risk, stratified by vascular risk factors

Quartiles	Cases	HR (95% CI) †	Cases	HR (95% CI) †	*P* for interaction
Sex	**Men**		**Women**		0.15
Q1	144	1.00	99	1.00	
Q2	117	1.05 (0.81–1.35)	142	1.10 (0.84–1.45)	
Q3	109	1.04 (0.80–1.36)	167	1.37 (1.04–1.80)	
Q4	152	1.15 (0.90–1.47)	139	1.41 (1.06–1.86)	
P for trend*		0.72		0.03	
BMI	**BMI < 23 kg/m^2**		**BMI ≥ 23 kg/m^2**		0.48
Q1	114	1.00	129	1.00	
Q2	104	0.92 (0.70–1.22)	155	1.18 (0.92–1.50)	
Q3	102	1.02 (0.76–1.37)	174	1.38 (1.08–1.77)	
Q4	107	1.09 (0.82–1.44)	184	1.41 (1.11–1.79)	
P for trend*		0.72		0.02	
Smoking Status	**Non-/Former Smoker**		**Current Smoker**		0.83
Q1	181	1.00	62	1.00	
Q2	198	1.10 (0.89–1.36)	61	0.95 (0.65–1.38)	
Q3	210	1.30 (1.05–1.61)	66	0.96 (0.66–1.40)	
Q4	207	1.35 (109.-1.67)	84	1.06 (0.74–1.51)	
P for trend*		0.02		0.91	
Alcohol	**Never/Monthly Drinker**		**Weekly/Daily Drinker**		0.49
Q1	207	1.00	36	1.00	
Q2	231	1.06 (0.87–1.29)	28	1.14 (0.67–1.91)	
Q3	254	1.23 (1.01–1.50)	22	1.03 (0.58–1.82)	
Q4	259	1.28 (1.05–1.56)	32	1.12 (0.67–1.87)	
P for trend*		0.04		0.96	
Diabetes	**No Diabetes**		**Diabetes**		0.03
Q1	134	1.00	109	1.00	
Q2	148	1.15 (0.90–1.47)	111	0.93 (0.70–1.22)	
Q3	130	1.08 (0.83–1.40)	146	1.30 (0.99–1.70)	
Q4	169	1.41 (1.10–1.80)	122	1.04 (0.79–1.38)	
P for trend*		0.03		0.05	
Hypertension	**No Hypertension**		**Hypertension**		0.65
Q1	140	1.00	103	1.00	
Q2	148	1.07 (0.84–1.36)	111	1.03 (0.78–1.37)	
Q3	175	1.32 (1.03–1.68)	101	1.03 (0.77–1.39)	
Q4	181	1.32 (1.04–1.67)	110	1.18 (0.88–1.57)	
P for trend*		0.04		0.67	

*Linear trend was tested using the median values of quartiles as a continuous variable in Cox proportional hazards models

†Hazard ratio (HR) adjusted for age at recruitment (year), year of study enrolment (1993–1995, 1996–1998), dialect group (Hokkien, Cantonese), educational level (no formal education, primary school, secondary school or higher), weekly physical activity (< 0.5 h/week, 0.5-<4 h/week, ≥ 4 h/week), total energy intake (kcal/day), intake of fruits and vegetables in quartiles, intake of poultry in quartiles, intake of fish/shellfish in quartiles, plus sex (men, women), BMI (< 23 kg/m^2, ≥ 23 kg/m^2), smoking (never/former, current), alcohol consumption (never/monthly, weekly/daily), history of diabetes (yes, no), history of hypertension (yes, no), and history of CAD/stroke (yes, no), except the factor being stratified

between red meat intake and PAD or CLTI risk. Despite differences in diet and food preparation methods across populations, our results concur with the other studies, and strengthen the observation that red meat consumption at high levels may increase the risk of PAD. In addition, it is also the first cohort to limit the study to severe disease requiring surgical intervention, while all other cohorts studying PAD have included a wide diversity of PAD ranging from subclinical disease to CLTI. As the morbidity and mortality burden of severe PAD is far greater than asymptomatic disease that can be treated conservatively, this study has further strengthened epidemiologic evidence that advocates for the reduction of red meat intake in patients with PAD and in the general population at large.

Diabetes is well-established as a very strong risk factor of CLTI worldwide [25–27]. The Oxford Vascular Study cohort, which followed up 92,728 subjects for 10 years, reported that diabetes increased the risk of critical limb ischemia (the old name for CLTI) [1] by 5.96 (95% CI = 3.15–11.26) [25]. In the United States, a retrospective study of 9 million individuals revealed that diabetes

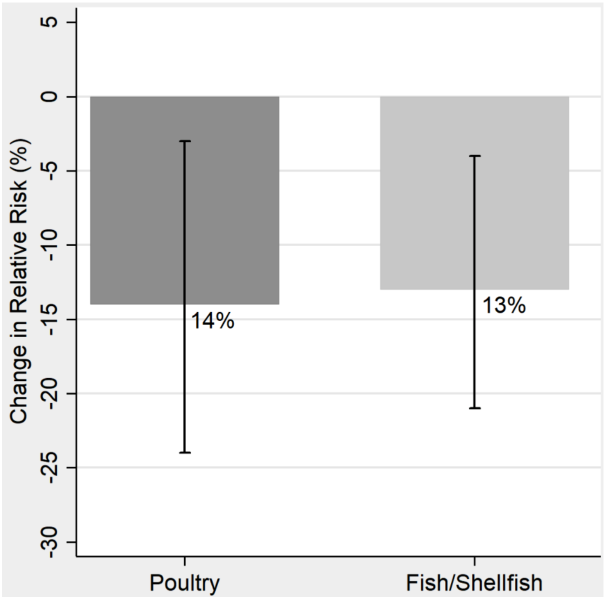

Fig. 2 Change in relative risk after substituting 3 servings/week of red meat with poultry or fish/shellfish

increased the odds of developing critical limb ischemia by 7.45 (95% CI=7.19–7.72) in subjects who did not have PAD [26]. In the present cohort, diabetes was previously reported to confer a 13-fold increase in risk of CLTI (HR=13.41; 95% CI=11.38–15.79) [27]. As red meat intake is also associated with the development of diabetes, both in populations worldwide according to a recent meta-analysis of 27 studies and in our cohort specifically, [28, 29] we postulate that the association between red meat and CLTI risk could be mediated via the presence of diabetes. Hence, it is not surprising that the association between red meat and CLTI risk could be weaker in those who already had diabetes at recruitment.

There are several biological mechanisms that may explain our findings. Compared to poultry or fish, red meat contains higher amounts of saturated fats and heme iron, both of which have been linked to atherosclerosis [8]. Saturated fatty acids are known to increase levels of low-density lipoprotein (LDL) cholesterol in the body, [30] which then undergoes oxidation, acetylation, and aggregation to generate modified LDL (mLDL). Macrophages then take up the mLDL to become foam cells and accumulate within the subendothelial space to form fatty streak lesions, which leads to atherosclerosis [31, 32]. This foam cell accumulation is further accelerated by high levels of heme iron in the lesion, [33] and animal studies have demonstrated that restricting dietary iron attenuates atherosclerosis progression in mice with iron overload phenotypes. [34] Another compound found in red meat, L-carnitine, is converted into trimethylamine-N-oxide (TMAO) by intestinal microbiota, [35, 36] and elevated plasma levels of TMAO has been associated with PAD in several cohort studies [36–39]. In the endothelium, TMAO induces vascular inflammation and enhances both the recruitment of macrophages into the endothelium as well as the mLDL-induced formation of foam cells [40, 41]. As such, the combination of atherogenic compounds in red meat may lead to accelerated atherosclerotic plaque formation and hence CLTI.

The main strengths of this study include the prospective design with a large sample size recruited from the general population and a long follow-up period with virtually complete data capture, which allows for modest associations to be detected and limits the risk of temporal bias and reverse causality. We comprehensively captured dietary information through in-person interviews using a 165-item FFQ that had been specifically developed for this cohort and demonstrated to be both internally consistent and reproducible [18]. The main limitation of this study is the use of dietary information from a single time point captured about 20 years prior to analysis, as it is possible that dietary habits may have changed across the follow-up duration. However, given the prospective design of the study, any misclassifications arising from the above limitations would likely be non-differential and result in the observed associations trending towards null [42]. Other limitations of this study are related to the observatory nature of the study and the use of self-reports for covariates in the models. In addition, the generalizability of this study may also be limited by the high consumption of pork in this population, [16] and hence our results may not be generalizable to other populations that commonly consume more beef or lamb. We did not collect any biomarkers as part of this study and were hence not able to directly measure inflammatory biomarkers of atherosclerosis. Finally, it is important to note that the substitution results in the present study are derived from theoretical modelling and not actual interventions.

In conclusion, in this large prospective population-based Asian cohort, high consumption of red meat was associated with increased CLTI risk, and this risk could be reduced by substituting red meat with other types of meat such as poultry or fish/shellfish. The findings in this study thus support dietary guidelines to reduce overall consumption of red meat to reduce the risk of severe vascular events.

Abbreviations

CAD	Coronary artery disease
CI	Confidence interval
CLTI	Chronic limb-threatening ischemia
FFQ	Food frequency questionnaire
HR	Hazard ratio
ICD	International Classification of Disease
LEA	Lower extremity amputation
PAD	Peripheral arterial disease

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12937-024-00991-9.

Supplementary Material 1

Acknowledgements

We are grateful to Ms. Siew-Hong Low of the National University of Singapore for supervising the fieldwork of the Singapore Chinese Health Study. We also thank the Ministry of Health in Singapore for assistance with the identification of CLTI cases via record linkage analysis with various databases.

Author contributions

W-PK and AFY designed and conducted the research, AFY and MT analyzed the data and all authors interpreted the analysis; AFY wrote the paper with critical input from W-PK; all authors contributed to the revision and approved the final manuscript. W-PK has primary responsibility for final content.

Funding

This work was supported by the United States National Cancer Institute, National Institutes of Health (UM1 CA182876 and R01 CA144034). MT was supported by Barts Charity, UK (MGU0570). DJH was supported by the National Medical Research Council, Singapore (MOH-STaR21jun-0003). W-PK was supported by the National Medical Research Council, Singapore [CSA-SI (MOH-000434)].

Data availability

The dataset used in this study is available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the institutional review board at the National University of Singapore (NUS-IRB), reference code LH-17-058.

Human ethics and consent to participate

Informed consent was obtained from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 25 January 2024 / Accepted: 31 July 2024
Published online: 07 September 2024

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