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Full Title : Red Meat Consumption and the Risk of Stroke: A Dose-Response Meta-Analysis of Prospective Cohort Studies

Short Title : Meat and Stroke

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Abbreviations :

CVD, cardiovascular disease

CHD, coronary heart disease

HR, hazard risk

RR, relative risk

OR, odds ratios

CI, confidence intervals

CI: Cerebral infarction

HS: Hemorrhagic stroke

IS: Ischemic stroke

Abstract

Background: Prospective studies of red meat consumption and risk of stroke have provided inconsistent results.

Objective: We aimed to assess this association by conducting a meta-analysis of prospective cohort studies.

Methods: Relevant studies were identified by searching PubMed and EMBASE through 1 April 2013. Summary relative risks (RR) and the corresponding 95% confidence intervals (CIs) were estimated by random-effect or fixed-effect models.

Results: Seven prospective cohort studies were included in the analyses, involving 2,079,236 subjects and 21,730 strokes cases. Total red meat consumption was associated with total stroke, cerebral infarction and ischemic stroke (RR=1.14, 95%CI [1.05, 1.24], RR=1.13, 95%CI [1, 1.28], RR=1.22, 95%CI [1.01, 1.46], respectively). A significant association between processed red meat and total stroke (RR=1.17, 95%CI [1.09, 1.27]). Fresh red meat was significantly associated with total stroke and ischemic stroke (RR=1.13, 95%CI [1.04, 1.22], RR=1.15, 95%CI [1.03, 1.29], respectively). However, no evidence suggests that any type of meat was associated with hemorrhagic stroke. Processed red meat and ischemic stroke (RR=1.15, 95%CI [0.98, 1.36]), fresh red meat and cerebral infarction (RR=1.06, 95%CI [0.94, 1.20]) also had no association between both. When the consumption of total red meat was above 50g/day, while processed red meat was just above 0g/day, and fresh red meat was above 70g/day, a significant risk for total stroke could be observed.

Conclusions: Our findings indicate that high consumption of red meat, especially processed red meat will increase the risk of stroke.

Keywords: meat; stroke; prospective studies; meta-analysis; dose-response

1. Introduction

As the global population aging process accelerating, stroke brings great harm to middle aged and elderly people, which aggravate the patient's family and socioeconomic burden. According to the American Heart Association report, each year 795,000 people experience a new or recurrent stroke and every 4 minutes someone dies from the disease¹. Owing to the high incidence and high mortality of stroke, the medical community ranks it as one of three top diseases that threats human health, along with coronary heart disease and cancer²⁻⁴.

Strokes can be classified into two major categories: ischemic and hemorrhagic, and ischemic stroke is the predominant type accounting for 87% of all cases⁵. Ischemic stroke can also be divided into two main types: thrombotic and embolic. A thrombotic stroke clinically referred to as cerebral thrombosis or cerebral infarction, and this type of event is responsible for almost 50 percent of all strokes. Owing to the category difference between the cerebral infarction and the ischemic stroke, it can be inferred that some factors that can cause ischemic stroke are not necessarily can cause cerebral infarction, so stroke can also be divided into three types: cerebral infarction, hemorrhagic stroke and ischemic stroke.

Meat consumption accounts for a large part of the diet and meat acts as a major source of protein, fat and energy for humans. However, the 2005 US Dietary Guidelines for Americans recommend that consumption of red and processed meat should be moderated⁶. Such recommendations are in large part derived from epidemiological evidence that high consumption of red meat was associated with increased risk of type 2 diabetes, digestive system disease, cancer and cardiovascular disease⁷⁻¹⁰. Recently, red meat consumption as a potential risk for stroke has been studied in two observational studies^{11, 12}. However, sufficient evidence for direct

relationships with stroke has been lacking to support more quantitative recommendations about specific consumption levels of meats.

Accordingly, we performed a systematic review and a meta-analysis of prospective studies to assess the relation between consumption of red meat (fresh red meat, processed meat, and total red meat) and the risk of total stroke and stroke subtypes, which could not be addressed in the previous meta-analysis.

2. Subjects and methods

2.1 Search strategy

We performed the meta-analysis on the basis of guidance of Meta-analysis of Observational Studies in Epidemiology. we performed a comprehensive literature search on PubMed database through April 1st 2013, by using the key words such as ‘meat’ ‘red meat’ ‘processed meat’ ‘meat products’ ‘beef’ ‘pork’ ‘lamb’ ‘steak’ ‘mutton’ ‘cardiovascular disease’ ‘hemorrhagic stroke’ ‘ischemic stroke’ ‘mortalities’ ‘risk’ ‘death’ ‘fatal’ ‘incidence’ (Figure1). In addition, we reviewed the reference lists of the published articles to identify additional relevant studies. To get additional data or main results for the meta-analysis, we contacted authors of primary studies (Table 1)¹³⁻¹⁹

“Red meat” was defined as unprocessed beef, pork and lamb except for poultry, fish and eggs; “processed meat” was mainly processed red meat like bacon, baloney, salami, hot dog and luncheon meats except for fish and eggs; “total red meat” was defined as the total of these two categories^{20, 21}. When possible, we conducted linear dose–response analysis of stroke risk per increment in consumption of 100 g/day for red meat, and 50 g/day for processed meat using generalized least-squares trend

estimation (GLST) analysis developed by Greenland and Orsini²².

2.2 Inclusion criteria

Studies were included if these (1) had a prospective cohort design; (2) defined the exposure factors are consumption of red meat, processed meat or fresh meat (unprocessed meat); (3) regarded different types of stroke as primary outcome indicators; (4) presented the hazard risk (HR), relative risk (RR) or odds ratios (OR) estimates with 95% confidence intervals (CIs); (5) regulated all participants are middle aged and elderly people.

2.3 Exclusion criteria

(1) Exclude other language articles except English; (2) Eliminate the repetitive articles or data; (3) some articles like comment, letter, review, editorial and animal studies were removed.

2.4. Data extraction and quality evaluation

The following data were extracted from each study: author's name, publication year, the experiment design, study location, age range or mean age of the participants, gender, number of cases and cohort size, length of follow-up, exposure range, RR with 95%CI of total strokes and stroke subtypes for each category of meat consumption. If the exposure factors include all types of total meat, we would only select the related information of total red meat and red meat types. If studies contain total stroke, stroke types, strokes morbidity and mortality, we prefer to choose the number of cases, the largest sample and the amount of information of the most complete data. Study selection and data extraction were conducted independently by

2 investigators (Yang and Pan), with disagreements resolved by consensus.

We used the Newcastle Ottawa Scale nine-star system to evaluate the study quality. The scoring system summarized 9 aspects of each study: representativeness of the exposed cohort, selection of the unexposed cohort, ascertainment of exposure, outcome of interest not present at start of study, control for important factor or additional factor, outcome assessment, follow-up time long enough for outcomes to occur, adequacy of follow-up of cohorts. Therefore, the full score was 9 stars, and a study with 7 awarded stars was defined the high-quality study²³.

2.5. Statistical analysis

2.5.1 All analyses were performed using Stata, version 11.0 (StataCorp, College Station, TX). The extracted RRs with 95% CI (highest compared to lowest red meat consumption) were computed from adjusted RRs, HRs, and ORs (HR and OR were directly considered as RR) to measure the association between red meat consumption and the risk of strokes.

2.5.2 The heterogeneity between the studies was assessed using Q and I² statistics. Because there are multiple factors such as participants, outcome indicators, methodology may affect the outcomes, we need to do the heterogeneity test, and if the heterogeneity is significant, we need to explore its source. For the Q statistic, a P value<0.10 was considered statistically significant for heterogeneity; for the I² statistic, heterogeneity was interpreted as absent (I²: 0%–25%), low (I²:25.1%–50%), moderate (I²: 50.1%–75%), or high (I²: 75.1%–100%), if I²≥ 50%, we need to choose random-effect model to indicate the obvious heterogeneity^{24, 25}.

2.5.3 A quantified dose-response relationship between total red meat, processed red meat, fresh meat consumption and incidence of strokes was examined on the basis of the principle of Greenland and Longnecker²¹. The analysis used data from each category of average consumption, number of cases and person years, and adjusted RR with its 95% CI. Forest plots and funnel plots were used to examine the overall effect and assess the publication bias^{26, 27}.

3. Results

3.1 Literature search and study characteristic

There were total 1,467 citations identified through the primary search. All studies were adjusted for age, smoking, fish, fruits, vegetables, BMI and disease history. Moreover, most studies also presented results on total energy consumption, physical activity, education level and alcohol. After the Newcastle Ottawa Scale nine star system analyses, the included articles reached 7stars, the 7 identified investigations were prospective cohorts. These studies were conducted in US (3 articles)^{13, 16, 19}, Sweden (2 articles)^{14, 15}, China (1 article)¹⁸ and Japan (1 article)¹⁷. A total of 2,079,236 unique individuals, including 21,730 stroke cases were involved (Table 1). The holistic heterogeneity was low ($I^2=0\%-54.5\%$) and there was no indication of a publication bias, either from Egger's test ($P= 0.780$) or from Begg's test ($P>0.05$ for both tests).

3.2. Total red meat consumption and strokes

The meta-analysis of total red meat intake and total stroke risk was based on four prospective cohort studies. In the highest versus lowest model, we found that the

summary RR was 1.14, 95% CI (1.05, 1.24). Heterogeneity test was conducted which shows no heterogeneity ($I^2=0$, $p=0.504$) (Figure 2-A). The RR between total red meat consumption and cerebral infarction was 1.13, 95% CI (1.0, 1.28), and the heterogeneity test suggests there was no statistical significance ($I^2=19.8\%$, $p=0.264$) (Figure 2-B). The RR between total red meat and hemorrhagic stroke was 1.18, 95% CI (0.93, 1.50), the results of heterogeneity test indicates the heterogeneity was moderate among studies (Figure 2-C). The RR between total red meat and ischemic stroke was 1.22, 95% CI (1.01, 1.46). Heterogeneity test was conducted and no heterogeneity among studies ($I^2=0\%$, $p=0.534$) (Figure 2-D).

3.3. Processed red meat consumption and strokes

Four prospective cohort studies are included in the highest versus lowest model, the meta-analysis of processed red meat intake and total stroke risk yielded a summary RR of 1.17, 95%CI (1.09, 1.27). No heterogeneity was detected ($I^2=0\%$, $p=0.464$) (Figure 2-A). The RR between processed red meat consumption and hemorrhagic stroke was 1.15, 95%CI (0.92, 1.44). The heterogeneity test was performed ($I^2=18.6$, $p=0.297$) (Figure 2-C) which indicates that there was no significant heterogeneity among studies. The RR between processed red meat and ischemic stroke was 1.15, 95%CI (0.98, 1.36). Low heterogeneity was detected ($I^2=27.6\%$, $P=0.24$) (Figure 2-D).

3.4. Fresh red meat and strokes

Five prospective cohort studies of fresh red meat distinction were included in the meta-analysis. In the highest versus lowest model, the summary RR was 1.13, 95%CI

(1.04, 1.22), and heterogeneity was absent ($I^2=0\%$, $p=0.826$) (Figure 2-A). The risk between fresh red meat and cerebral infarction was 1.06, 95%CI (0.94, 1.20). No heterogeneity was detected ($I^2=0\%$, $p=0.451$) (Figure 2-B). The RR between fresh red meat and hemorrhagic stroke was 0.88, 95%CI (0.73, 1.06). Low heterogeneity was detected ($I^2=43.7\%$, $p=0.1$) (Figure 2-C). The RR between fresh red meat and ischemic stroke was 1.15, 95%CI (1.03, 1.29). No heterogeneity was detected ($I^2=0$, $p=0.552$) (Figure 2-D).

3.5. Dose-response meta-analysis

Three studies were combined to conduct the dose-response meta-analysis about red meat consumption and total stroke. Dose-response analysis indicated that there was no risk for total stroke if the total red meat consumption was below 50g/day, however, if it was above 50g/day, a significant risk can be observed (Figure 3-A). The consumption of processed red meat was suggested to be significantly associated with higher risk of total stroke if the dose was above 0 g/day (Figure 3-B). There was no risk for total stroke if the consumption of processed red meat was below 70 g/day, however, if it was above 70 g/day, it was related to total risk significantly (Figure 3-C)²².

3.6. Publication bias

Begg and Egger tests indicated no evidence of publication bias with regard to total red meat consumption in relation to the risk of total stroke (Figure 4).

4. Discussion

This meta-analysis of 7 prospective studies, including a large number of stroke cases, showed that the potential association between red meat consumption and stroke.

Consumption of total red meat was associated with total stroke, cerebral infarction and ischemic stroke. There was a significant association between processed red meat consumption and total stroke. Fresh red meat consumption was significantly associated with total stroke and ischemic stroke. However, not any statistically significant associations were observed for hemorrhagic stroke. Processed red meat and ischemic stroke, fresh red meat and cerebral infarction also had no association between both. When the consumption of the total red meat was above 50g/day, while processed red meat consumption was just above 0g/day, and fresh red meat was above 70g/day, a significant risk for total stroke could be observed in them.

The mechanism why much red meat consumption leads the stroke may be explained by several potential biochemical views. Red meat contains abundant heme iron, and heme iron can contribute to endogenous formation of N-nitroso compounds (NOC)²⁸, which has been linked to cardiovascular disease and stroke in epidemiological studies^{29, 30}. In experimental studies, nitrates and their byproducts have been suggested to promote atherosclerosis and vascular dysfunction³¹, reduce insulin secretion^{32, 33} all of which may contribute to increase the risk of stroke. And oxidative stress and DNA damage caused by iron is also thought to be a risk factor for stroke^{34, 35}. In addition, Red meats contain high amounts of saturated fatty acids and cholesterol which has been shown to have adverse effects on serum lipid profiles and subsequently may increase the risk of stroke³⁶⁻³⁸.

High consumption of red meat has been suggested to negatively impact on human health, but too less red meat consumption is also harmful. Red meat has been long established as an important dietary source of protein and essential nutrients including iron, zinc and vitamin B12^{39, 40}. As described above, red meat is a major source of heme iron. Iron is vital for many cellular processes in the body and, as a component of hemoglobin, is essential to maintaining adequate transport of oxygen in the blood²⁸. Iron-deficiency anaemia (IDA) is the most common nutritional deficiency worldwide, being particularly prevalent among children and young women⁴¹. And anemia is known to be one of the stroke causes⁴². All in all, moderate consumption of red meat as part of a balanced diet may positively influence nutrient intakes, while too less red

meat consumption will not be benefit for human long-term health.

The strength of our study lies in a large sample size (2, 079,236 subjects and 21,730 strokes cases) and no significant evidence of publication bias. Furthermore, all the original studies included in this meta-analysis were high-quality researches, and had a prospective cohort design. In addition, we used the nonlinear curve to analyze the dose-response which is more accurate and credible.

Although we have tried best to get more precise conclusions, there still exist several limitations. Firstly, most of population data were from questionnaire, while different questionnaire design has some loopholes. During the implementation of questionnaire, the number of participants is so large that the questionnaire is difficult to spread, and thus questionnaire quality is not guaranteed. Secondly, the lifestyle plays an important role. Unhealthy lifestyle such as smoking, alcohol, physical inactivity, and overeating salty may affect the reliability of results. These confounding factors tend to exaggerate the risk between red meat and stroke^{43, 44}. Next, some diseases like heart diseases, diabetes mellitus and hypertension will increase the risk of stroke. Heart disease patients are prone to have thrombus, which may cause a stroke; diabetes patients not only are prone to develop blood lipid metabolic disorders, aggravate atherosclerosis, but also are prone to suffer from hypertension, which are risk factors for stroke; once hypertensive patients whose blood pressure suddenly change, it will lead to a stroke⁴⁵⁻⁴⁷. Lastly, during the long follow-up, participants may have changed their diets. They may adjust to a healthy diet structure in which the red meat consumption was reduced. All in all, these potential factors are likely to impact the results.

5. Conclusion

In summary, our meta-analysis supports that the consumption of fresh red meat, processed red meat and total red meat has significant risk for total stroke and ischemic stroke except hemorrhage stroke. As to cerebral infarction, it has not been clearly confirmed. In addition, the dose-response analysis demonstrates that the consumption of processed red meat and total red meat rather than fresh red meat is consistently associated with the risk of stroke in a nonlinear fashion. These results indicate that high consumption of red meat, especially processed red meat will increase the risk of stroke.

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Figure legends

Figure1. Literature searched and selection of studies in the meta-analysis.

Figure2. Estimates of red meat consumption and risk of stroke

- (A) Estimates of red meat consumption and risk of total stroke.
- (B) Estimates of red meat consumption and risk of cerebral infarction.
- (C) Estimates of red meat consumption and hemorrhagic stroke risk.
- (D) Estimates of red meat consumption and ischemic stroke risk.

Squares indicated study-specific risk estimates (size of square reflects the study-statistical weight, i.e. inverse of variance); horizontal lines indicate 95% CI; diamond indicates summary relative risk estimate with its corresponding 95% CI.

Figure 3. Dose-response analysis.

A. Dose-response analysis of stroke risk and total red meat intake by per increment of 100 g/day for red meat, and 50 g/day for processed meat.

B. Processed red meat and total stroke risk dose-response analysis.

C. Fresh red meat and total stroke dose-response analysis.

The solid line and the long dash line represent the estimated relative risk and its 95% CI. Short dash line represents the linear relationship.

Figure 4. Analysis of publication bias about total red meat and total stroke.

A. begg's funnel plots to detect potential publication bias about total red meat and total stroke

B. egger's funnel plots to detect potential publication bias about total red meat and total stroke.

Author	location, study type/follow years	NO. of participant/ follow years/age	meat type	Stroke type	RR for strokes	dose	Adjustment
Bernstein	US Prospective cohort Men/21 year Women/26 year	1,397 total strokes, 829 ISs, 165 IC, 53 HS (43,150), MEN Age 40-75 2,633 total strokes, 1,383 ISs, 235ICHs, 240 HS (84,010) WOMEN Age 30-55	Total red meat(man)	total stroke	1.28(1.02-1.61)		Age, body mass index, cigarette smoking,
				HS	1.07(0.55,2.08)		physical
			total red	IS	1.31(0.97,1.77)		Exercise, parental history of early myocardial
			meat (women)				infarction, menopausal status, total energy,
				total stroke	1.19(1-1.41)		cereal fiber, trans fat, fruit and vegetables,
				HS	1.30(0.72,2.34)		other
				IS	1.16(0.92,1.48)		protein sources
			Processed	total stroke	1.27 (1.03–1.55)		
			red meat(man)	HS	1.47 (0.80,2.72)		
				IS	1.31 (1.00,1.71)		
			Processed	total stroke	1.10 (0.95–1.27)		
			red meat(woman)	HS	0.94 (0.56,1.57)		
				IS	1.07 (0.87,1.31)		
Larsson (man)	Swedish	Total red meat	fresh red meat(man)	total stroke	1.11 (0.88 –1.39)		
				HS	0.70 (0.36,1.37)		
				IS	1.23 (0.91,1.67)		
			fresh red meat(woman)	total stroke	1.19 (1.02–1.40)		
				HS	0.93 (0.54,1.60)		
				IS	1.30 (1.03,1.63)		
Larsson (man)	Swedish	Total red meat	Total stroke		1.15 (1.00, 1.33)	(total red meat)	

Larsson (woman)	Swedish Prospective cohort 10.4 year	1680 incident cases of stroke, comprising 1310 cerebral infarction, 154 intracerebral hemorrhage, 79 subarachnoid hemorrhage, and 137 unspecified stroke (34670) age 49-83	Total red meat	Total stroke	1.12 (0.95–1.32)	Total red meat		age, smoking status and pack-years of smoking, education, body mass index, total physical activity, history of diabetes, history of hypertension, aspirin use, family history of myocardial infarction, and intake of total energy, alcohol, coffee, fish, fruits, and vegetables.						
						CI	1.22 (1.01–1.46)		<36.5	36.5 – 53.6				
											IS	0.74(0.45,1.12)	53.7 – 68.3	68.4 – 85.9
						fresh Red meat	Total stroke		1.07 (0.91–1.23)	(Fresh red meat and processed red meat)				
												CI	1.12 (0.93–1.34)	
														HS
						Processed meat	Total stroke		1.18 (1.00–1.38)	36.5 – 48.7	≥48.8			
												HS	0.91(0.6,1.39)	
He ka	Us, prospective cohort/ 14 year	455 ischemic strokes, 125 hemorrhagic stokes, and 145 strokes of unknown type , (51 529) age 40-75	Total Red meat	Total stroke	1.58 (0.55 to 4.55)			body mass index , physical activity , history of hypertension , smoking status , aspirin use , multivitamin use , and consumption of alcohol , potassium, fiber, and Vitamin E, total fruit and vegetables, total energy intake, and hypercholesterolaemia						
						HS	0.97(0.60, 1.55)							

Takata	prospective cohort/ 11.2 year	women and 334,281 men age 40-70	Red meat(women)	HS	0.57 (0.37, 0.87)	(Total red meat fresh and processed red meat)		age , total caloric intake, income, occupation, education, comorbidity index, physical activity level, total vegetable intake, total fruit intake, fish intake, appropriate, smoking history , alcohol
				IS	0.84 (0.55, 1.28)			
			Red meat(men)	HS	0.71 (0.43, 1.20)			
				IS	1.22 (0.69, 2.15)			
Sauvage	Japan prospective cohort /16 year	1462 stroke deaths 15,350 men and 24,999 women Mean age 56	Red meat	Total stroke	1.01 (0.73, 1.38)	(Total red meat fresh and processed red meat)		city, radiation dose, self-reported body mass index, smoking status, alcohol habits, education level, history of diabetes, or hypertension
			Processed red meat	Total stroke	0.90 (0.61, 1.33)			
Sirin Yaemsiri	US and Columbia prospective cohort / 8 year	1,049 ischemic stroke (663,041) Age 50-79	Total red meat	Total stroke	0.95 (0.75,1.23)	(Total red meat fresh and processed red meat)		age and race, education, family income, smoke, total metabolic equivalent task hours per week, alcohol intake, history of coronary heart disease, history of atrial fibrillation, history of diabetes, aspirin use, use of antihypertensive medication, use of cholesterol-lowering medication, body mass index, systolic blood pressure, and total energy intake, vitamin E, fruits and vegetable intake (quintiles), fiber
			Red meat	IS	1.13 (0.95–1.34)			

Table 1. Characteristics of observational studies of the relation between read meat intakes and risk of stroke included in the meta-analysis.

CI: Cerebral infarction, HS: Hemorrhagic stroke, IS: Ischemic stroke.

RRs: include RR/HR (Song/ Knekt: RR, Wedick/Nettleton: HR).

Figure 1. Literature searched and selection of studies in the meta-analysis.

A

B

C

D

Figure 2. Estimates of red meat consumption and risk of stroke.

A. Dose-response analysis of stroke risk and total red meat intake by per increment of 100 g/day for red meat, and 50 g/day for processed meat.

B. Processed red meat and total stroke risk dose-response analysis.

C. Fresh red meat and total stroke dose-response analysis.

Figure 3. Dose-response analysis.

A. begg's funnel plots to detect potential publication bias about total red meat and total stroke.

B. egger's funnel plots to detect potential publication bias about total red meat and total stroke.

Figure 4. Analysis of publication bias about total red meat and total stroke.