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ORIGINAL ARTICLE

Meat consumption in relation to mortality from cardiovascular disease among Japanese men and women

M Nagao[1,2], H Iso[1], K Yamagishi[3], C Date[4] and A Tamakoshi[5] for the JACC Study Group

BACKGROUND/OBJECTIVES: Although high or low (no) meat consumption was associated with elevated or reduced mortality from cardiovascular disease, respectively, few studies have investigated the association between moderate meat consumption and cardiovascular disease. We aimed to evaluate the associations between moderate meat consumption and cardiovascular disease mortality.

SUBJECTS/METHODS: We conducted a prospective cohort study of 51 683 Japanese (20 466 men and 31 217 women) aged 40 - 79 years living in all of Japan (The Japan Collaborative Cohort Study; JACC Study). Consumptions of meat (beef, pork, poultry, liver and processed meat) were assessed via a food frequency questionnaire administrated at baseline survey. Hazard ratios (HRs) of mortality from cardiovascular disease were estimated from Cox proportional hazards regression models according to quintiles of meat consumption after adjustment for potential confounding variables.

RESULTS: During 820 076 person-years of follow-up, we documented 2685 deaths due to total cardiovascular disease including 537 ischemic heart diseases and 1209 strokes. The multivariable HRs (95% confidence interval) for the highest versus lowest quintiles of meat consumption (77.6 versus 10.4 g/day) among men were 0.66 (0.45 - 0.97) for ischemic heart disease, 1.10 (0.84 - 1.43) for stroke and 1.00 (0.84 - 1.20) for total cardiovascular disease. The corresponding HRs (59.9 versus 7.5 g/day) among women were 1.22 (0.81 - 1.83), 0.91 (0.70 - 1.19) and 1.07 (0.90 - 1.28). The associations were similar when the consumptions of red meat, poultry, processed meat and liver were examined separately.

CONCLUSION: Moderate meat consumption, up to ~100 g/day, was not associated with increased mortality from ischemic heart disease, stroke or total cardiovascular disease among either gender.

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Keywords: meat; cardiovascular diseases; ischemic heart disease; stroke; mortality; prospective study

INTRODUCTION

Meat is a major protein source for humans.[1] Unlike fish or beans, however, meat in particular red or processed meat is often considered to be unhealthy because of the fact that it is a major source of saturated fatty acid (a reported risk factor for ischemic heart disease in Western countries).[2-4] Several cohort studies have reported an association between high consumption of meat (which is two to three more times likely in Westerners but not in Japanese) in particular red or processed meat and risk of ischemic heart disease[5-7] and stroke,[8,9] while one Japanese study showed no association between meat consumption and stroke mortality.[10] The evidence on low (no) meat consumption and cardiovascular disease came from studies of vegetarians,[11-15] although their diet and lifestyles may differ in many ways to that of non-vegetarians. Studies of non-vegetarians or general populations are necessary to evaluate an effect of low-to-moderate meat consumption on cardiovascular disease, but no studies have examined this issue. In recent decades, the average meat consumption among Japanese increased gradually during the 1990s, and stabilized thereafter (64.2 g/day for total meat in 1975, 82.3 g/day in 1995 and 82.6 g/day in 2007).[1] However, meat consumption among Japanese remains much lower than that among people from Western countries (179.3 g/day for total meat).[16]

In this paper, we examined the association between moderate meat consumption and mortality from cardiovascular disease in a large prospective study of Japanese men and women.

SUBJECTS AND METHODS

Study cohort

The Japan Collaborative Cohort (JACC) Study is a population-based study of 110 792 persons (46 465 men and 64 327 women) aged 40 - 79 years during the baseline period (1988 - 1990), enrolled from 45 communities across Japan. The sampling and protocols of the JACC Study have been described previously.[17] Participants replied to self-administered questionnaires about their lifestyles and medical histories such as cardiovascular disease and cancer.[18] We excluded persons who had a history of heart disease, stroke or cancer at the baseline survey ($n = 5864$), or participants with a missing response to more than four items on the food frequency questionnaire (FFQ; described below ($n = 46\,198$)).[19] Furthermore, participants with one or more item missing among five meat items listed on the dietary questionnaire were excluded ($n = 7047$). In addition, we obtained

[1]Public Health, Department of Social and Environmental Medicine, Osaka University, Graduate School of Medicine, Osaka, Japan; [2]Department of Public Health, Dokkyo Medical University School of Medicine, Shimotsugagun-Mibu, Japan; [3]Faculty of Medicine, Department of Public Health Medicine, University of Tsukuba, Tsukuba, Japan; [4]Department of Food Science and Nutrition, School of Human Science and Environment, University of Hyogo, Himeji, Japan and [5]Department of Public Health, Aichi Medical University School of Medicine, Aichi, Japan. Correspondence: Professor H Iso, Public Health, Department of Social and Environmental Medicine, Graduate School of Medicine, Osaka University, Suita, Osaka-fu 565-0871, Japan.
E-mail: iso@pbhel.med.osaka-u.ac.jp

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the data of serum total cholesterol levels at municipal health screening examinations from the subsamples of 5800 men and 11 291 women.

As a result, we included 20 466 men and 31 217 women who provided complete information regarding their dietary information. Written or explicitly verbal informed consent was obtained before their questionnaires were completed. In several communities, informed consent was obtained from community leaders on behalf of the individual participants (a common practice for informed consent in Japan at that time). The JACC Study protocol was approved by the Medical Ethical Committees of the Nagoya University School of Medicine, University of Tsukuba and Osaka University.

Mortality surveillance

In each community, investigators conducted a systematic review of death certificates. In Japan, the registration of residence and death is a legal requirement, so it is assumed that this practice is upheld. The participants moved out from their original community were treated as censored cases, for whom the dates of moving out were obtained from the registration database. Follow-up was conducted through to the end of 2008, except for four communities in which the follow-up had ended in 1999, and another four communities for which it had ended in 2003. The median follow-up period was 18.4 years. We used the underlying cause of death coded by the International Statistical Classification of Diseases and Related Health Problems–10th Revision (ICD-10) to identify mortality end points: I20–I25 for ischemic heart disease, I60–I69 for stroke and I01–I99 for total cardiovascular disease.

Consumption of meat

Dietary assessment was evaluated by the FFQ. The FFQ included 33 foods, including 5 meat items: beef, pork, poultry, liver and processed meat. Liver item was considered as meat in this study because its protein and fat contents are similar to lean meat. Five choices were presented for each item with regard to consumption habits: rarely, 1–2 days a month, 1–2 days a week, 3–4 days a week and almost daily. The portion size per day was estimated by a validation study for FFQ among 85 participants. We assigned portion size as 36 g for beef, 41 g for pork, 43 g for poultry, 22 g for processed meat and 53 g for liver.[19] In addition, we multiplied the portion size by 1.3 for men for the following two reasons. First, the portion size estimated from the validation study was primarily for women, because the majority of participants (77 out of 85) in the validation study were women. Second, average meat consumption was 1.3 times higher among men than women according to the National Nutrition Survey in 1990.[20] We calculated consumption of meat (g/day) from average daily frequencies and portion sizes. On the basis of the Japan Food Table (Fifth Revised and Enlarged Edition), we calculated the intake of total energy from frequency and portion size of each food.[19]

The reproducibility of this FFQ was confirmed by comparing two questionnaires administered 1 year apart in the validation study.[19] The Spearman correlation coefficients were 0.59 for beef, 0.44 for pork, 0.53 for poultry, 0.67 for processed meat and 0.71 for liver. Further, the validity of dietary data was confirmed by comparing the data from the questionnaire with those from four 3-day dietary records, collected approximately 3–4 months apart.[19] The Spearman correlation coefficients were 0.49 for beef, 0.37 for pork, 0.44 for poultry, 0.63 for processed meat and 0.20 for liver. The sex-specific mean values (standard deviations) of total meat consumption were 75.9 g (37.1) among men and 55.8 g (24.7) among women for dietary record, and 37.8 g (26.6) among men and 29.8 g (20.8) among women for FFQ.

Statistical analysis

Age-adjusted means and proportions of selected cardiovascular risk factors and foods were calculated according to quintiles of consumptions of total meat, red meat (beef and pork), poultry, processed meat and liver. Foods were adjusted for energy by the residual method.[21] For each participant, we calculated the person-years of follow-up from baseline to the first end point: death, moving from the community or the end of 2008. The mortality rates of each outcome were calculated according to quintiles of meat consumption. Gender-specific hazard ratios with 95% confidence intervals (CIs) were calculated after adjustments for age, and other potential confounding factors using Cox proportional hazards regression models. The statistical testing for linear trends across quintiles of meat consumption was performed by using a median value of each quintile. The confounding factors included body mass index (BMI; gender-specific quintiles), history of hypertension and diabetes mellitus (yes or no), smoking status (never, former, current smoker of 1–19 or $\geqslant$20 cigarettes/day),[22] alcohol intake (never, former drinker and current drinker of ethanol at 1–22, 23–45, 46–68 or $\geqslant$69 g/day), perceived mental stress (low, medium or high),[23] walking time (rarely, 30, 30–60 or more than 60 min/day),[24] sports participation time (rarely, 1–2, 3–4 or more than 5 h/week),[24] education levels (age of completed education of $<$13, 13–15, 16–18 or $\geqslant$19 years), total energy (gender-specific quintiles) and energy-adjusted food (rice, fish, soy, vegetable and fruit) intake (gender-specific quintiles). The self-reported weight and height figures were obtained from the baseline questionnaire. We analyzed this data using SAS version 9.1.3 Service Pack 4 (SAS Institute, Cary, NC, USA). All probability values for statistical tests were two-tailed, and values of $P<0.05$ were considered as statistically significant.

RESULTS

During 820 076 person-years of follow-up for 51 683 persons (20 466 in men and 31 217 in women), we documented 537 deaths (301 in men and 236 in women) due to ischemic heart disease, 1209 (589 in men and 620 in women) due to stroke and 2685 (1317 in men and 1368 in women) total cardiovascular deaths.

At baseline, median consumption of total meat was 33.7 g/day for men and 27.0 g/day for women. According to quintiles of energy-adjusted total meat consumption, history of diabetes mellitus was positively associated with total meat consumption. BMI, ethanol intake, blood pressure and history of hypertension were inversely associated with total meat consumption (Table 1). Similar trends were observed according to quintiles of red meat consumption and quintiles of poultry, processed meat and liver consumptions (not shown in table). In the subsample of 5800 men and 11 291 women, meat consumption was not associated with serum total cholesterol levels. For men, there were no associations between total meat consumption and age-adjusted mortality from stroke or total cardiovascular disease, although there was an inverse trend with mortality from ischemic heart disease (Table 2). After adjustment for cardiovascular risk factors and selected food intakes, the inverse association with mortality from ischemic heart disease remained statistically significant. The multivariable hazard ratios (95% CI) of mortality from ischemic heart disease for the highest versus lowest quintiles of total meat consumption was 0.66 ((0.45–0.97); P-trend = 0.015). For women, total meat consumption was not associated with mortality from ischemic heart disease or total cardiovascular disease in either age-adjusted or multivariable models. Although there was an inverse trend with mortality from stroke in age-adjusted model, the association was no longer statistically significant in multivariable model (Table 2).

The inverse association with ischemic heart disease was similarly observed for red and processed meat consumption among men, while no association for any subtype of meat consumption among women (Table 3).

Stratified by BMI (less and more than 25 kg/m^2), these associations did not alter except for overweight women (Table 4). Among overweight women, in which mean BMI was 27.1 kg/m^2, the multivariable hazard ratio (95% CI) of mortality from ischemic heart disease for highest versus lowest quintiles of total meat consumption was 2.04 ((0.98–4.26); P-trend = 0.040). The associations between total meat consumption and mortality from stroke or total cardiovascular disease did not vary according to the BMI subgroup among men and women.

The inverse association with ischemic heart disease was not observed for any meat subtype among overweight men, although it was observed for processed meat among non-overweight men

Table 1. Sex-specific age-adjusted mean values or prevalence of cardiovascular risk factors according to quintiles of total meat consumption[a]

Quintile of total meat consumption[b]	*Men*					P *for trend*[c]	*Women*					P *for trend*[c]
	Q1 (low)	*Q2*	*Q3*	*Q4*	*Q5 (high)*		*Q1 (low)*	*Q2*	*Q3*	*Q4*	*Q5 (high)*	
Men												
Meat consumption, median (g/day)	10.4	23.1	33.9	46.6	77.6		7.5	18.7	27.1	37.1	59.9	
Range (g/day)	(0.7 - 17.4)	(17.4 - 28.7)	(28.7 - 39.5)	(39.5 - 55.2)	(55.2 - 277.1)		(0.4 - 13.9)	(13.9 - 23.1)	(23.1 - 31.5)	(31.5 - 43.7)	(43.7 - 221.6)	
Meat consumption (times/day)	0.21	0.49	0.71	0.95	1.52		0.20	0.53	0.74	1.00	1.50	
Participants at risk (*n*)	4093	4093	4094	4093	4093		6243	6244	6243	6244	6243	
Age (years)	56.6	55.7	55.3	55.0	55.8	0.006	58.8	56.7	55.5	55.0	54.3	<0.001
BMI (kg/m^2)	22.8	22.8	22.7	22.6	22.5	<0.001	23.0	22.9	22.9	22.8	22.8	<0.001
Smoker (%)	56.5	54.0	53.4	52.2	54.5	0.078	6.1	4.6	4.3	4.2	4.7	0.003
Ethanol intake (g/day)	37.1	35.4	34.1	32.8	31.0	<0.001	12.8	11.6	9.6	9.0	8.0	<0.001
Walking time 30 min or more/day (%)	67.4	70.3	70.3	69.2	68.7	0.720	70.6	73.0	73.2	73.2	71.2	0.850
Sports time 1 h or more/week (%)	27.5	31.9	32.6	33.1	32.4	<0.001	19.6	22.1	25.0	24.8	24.8	<0.001
Sleep duration (h/day)	7.4	7.4	7.4	7.4	7.4	0.020	7.1	7.1	7.1	7.1	7.0	0.046
College or higher education (%)	18.2	18.1	19.2	18.0	19.3	0.256	8.5	9.6	11.5	11.1	11.5	<0.001
High perceived mental stress (%)	25.0	24.5	23.4	24.7	26.7	0.033	20.7	20.5	20.7	20.8	20.9	0.597
History of hypertension (%)	18.8	18.5	17.2	17.0	16.1	<0.001	20.2	19.7	18.8	18.4	17.6	<0.001
History of diabetes (%)	5.3	5.5	5.6	5.9	5.9	0.177	3.1	3.1	2.7	3.2	3.6	0.083
Menopause (%)	–	–	–	–	–	–	62.6	65.4	64.3	63.1	61.2	<0.001
Rice intake (g/day)[d]	571.1	539.6	515.1	490.6	441.5	<0.001	467.8	433.8	415.0	389.7	352.5	<0.001
Fish and fish products intake (g/day)[d]	44.4	46.4	48.8	51.0	57.4	<0.001	48.4	48.0	48.7	50.5	54.2	<0.001
Vegetables intake (g/day)[d]	80.3	88.2	93.5	98.9	110.0	<0.001	101.3	102.7	105.5	110.1	117.5	<0.001
Fruits intake (g/day)[d]	107.5	116.3	123.7	125.2	135.7	<0.001	142.4	146.9	149.8	153.8	155.0	<0.001
Energy intake (kcal/day)	1731	1811	1795	1766	1722	<0.001	1395	1487	1452	1430	1367	<0.001
Systolic blood pressure (mmHg)[e]	134.7	133.5	133.5	133.0	132.3	<0.001	131.1	130.4	130.0	130.1	129.7	<0.001
Diastolic blood pressure (mmHg)[e]	81.0	80.0	80.1	80.1	79.3	<0.001	77.0	77.0	76.9	77.0	76.8	0.211
Subsamples with serum chemistry (*n*)	1133	1226	1186	1126	1129		2092	2181	2285	2283	2450	
Total cholesterol (mmol/l)	4.90	4.90	4.94	4.87	4.92	0.837	5.27	5.30	5.28	5.27	5.25	0.200

[a]Meat consumption was estimated as the frequency scores and portion size of five meat items (beef, pork, poultry, liver and processed meat). [b]Energy adjusted quintiles by residual method. [c]On the basis of the tests for trend across quintiles of meat consumption by assigning the median value of each quintile. [d]Food intakes were adjusted for total energy intake by the residual method. [e]Systolic blood pressure and diastolic blood pressure were obtained at baseline survey by self-reported.

Table 2. Sex-specific hazard ratios (HRs) and 95% confidence intervals (95% CIs) of mortality from ischemic heart disease, stroke and total cardiovascular disease according to quintiles of total meat consumption (energy adjusted)

	Quintile of total meat consumption					P *for trend*[a]
	Q1 (low) reference	*Q2 HR 95% CI*	*Q3 HR 95% CI*	*Q4 HR 95% CI*	*Q5 (high) HR 95% CI*	
Men (*n*)	4093	4093	4094	4093	4093	
Person-years	62 660	64 307	64 761	64 405	62 954	
Ischemic heart disease (*n*)	74	65	63	49	50	
Age-adjusted HR	1.00	0.90 (0.65 - 1.26)	0.89 (0.63 - 1.24)	0.72 (0.50 - 1.03)	0.70 (0.49 - 1.01)	0.027
Multivariable HR[b]	1.00	0.97 (0.69 - 1.36)	0.92 (0.65 - 1.30)	0.73 (0.50 - 1.06)	0.66 (0.45 - 0.97)	0.015
Total stroke (*n*)	130	105	122	101	131	
Age-adjusted HR	1.00	0.85 (0.66 - 1.10)	1.00 (0.78 - 1.28)	0.86 (0.66 - 1.11)	1.05 (0.82 - 1.33)	0.560
Multivariable HR[b]	1.00	0.91 (0.70 - 1.19)	1.08 (0.83 - 1.39)	0.92 (0.70 - 1.20)	1.10 (0.84 - 1.43)	0.461
Total cardiovascular disease (*n*)	300	260	246	233	278	
Age-adjusted HR	1.00	0.91 (0.77 - 1.07)	0.87 (0.74 - 1.03)	0.86 (0.72 - 1.01)	0.96 (0.82 - 1.14)	0.721
Multivariable HR[b]	1.00	0.98 (0.83 - 1.16)	0.94 (0.79 - 1.12)	0.93 (0.78 - 1.11)	1.00 (0.84 - 1.20)	0.988
Women (*n*)	6243	6244	6243	6244	6243	
Person-years	98 857	100 192	100 918	100 589	100 433	
Ischemic heart disease (*n*)	73	44	39	35	45	
Age-adjusted HR	1.00	0.80 (0.55 - 1.17)	0.79 (0.54 - 1.17)	0.73 (0.49 - 1.10)	1.00 (0.69 - 1.46)	0.851
Multivariable HR[b]	1.00	0.89 (0.60 - 1.30)	0.93 (0.62 - 1.40)	0.89 (0.58 - 1.35)	1.22 (0.81 - 1.83)	0.397
Total stroke (*n*)	185	138	100	103	94	
Age-adjusted HR	1.00	0.97 (0.78 - 1.21)	0.78 (0.61 - 1.00)	0.83 (0.65 - 1.06)	0.81 (0.63 - 1.04)	0.041
Multivariable HR[b]	1.00	1.02 (0.82 - 1.28)	0.88 (0.68 - 1.13)	0.92 (0.72 - 1.19)	0.91 (0.70 - 1.19)	0.357
Total cardiovascular disease (*n*)	407	265	233	232	231	
Age-adjusted HR	1.00	0.86 (0.74 - 1.01)	0.84 (0.72 - 0.99)	0.87 (0.74 - 1.02)	0.92 (0.78 - 1.08)	0.288
Multivariable HR[b]	1.00	0.91 (0.77 - 1.06)	0.93 (0.79 - 1.10)	0.98 (0.83 - 1.16)	1.07 (0.90 - 1.28)	0.351

[a]On the basis of the tests for trend across quintiles of meat consumption by assigning the median value of each quintile. [b]Cox proportional hazard models adjusted for age, body mass index, ethanol intake, perceived mental stress, walking time, sports participation time, education years, history of hypertension and diabetes, total energy and energy-adjusted food (rice, fish, soy, vegetables and fruits) intakes.

(Supplementary Table 1). For women, the positive trend was observed for liver consumption among the overweighted, while no association for any meat subtype was shown among the non-overweighted women (Supplementary Table 2).

To examine a potential reverse causation for meat consumption and mortality from cardiovascular disease, we analyzed the data by excluding early deaths. When deaths occurring 1–8 years from the baseline (the middle of the follow-up period) were excluded, the associations between total meat consumption and mortality from ischemic heart disease did not change substantially. For example, after the exclusion of deaths within 8 years from the baseline, the multivariable hazard ratios (95% CI) of ischemic heart disease mortality for highest versus lowest quintiles of total meat consumption were 0.69 ((0.44–1.09); *P*-trend = 0.058), among men, and 1.19 ((0.75–1.90); *P*-trend = 0.57), among women. The associations between red meat, processed meat, poultry or liver with mortality from ischemic heart disease did not change by excluding early deaths.

DISCUSSION

In this large, community-based prospective cohort study, we observed that consumption of total meat was not associated with increased risk of mortality from stroke and total cardiovascular disease. Persons with higher meat consumptions comparing to those with the lowest consumption of total meat, red meat and processed meat were associated with lower risk of mortality from ischemic heart disease for men, while no associations were found for women. Among overweight women (BMI $\geqslant 25\,kg/m^2$), however, the highest quintile of meat consumption tended to be associated with the elevated risk.

Japanese meat consumption increased from 64.2 g/day in 1975 to 82.3 g/day in 1995, stabilizing at 82.6 g/day in 2007.[1] Age-adjusted mortality rates from ischemic heart disease declined from 46.3 per 100 000 in 1990 to 38.7 per 100 000 in 2007 for men, and from 25.6 per 100 000 to 17.0 per 100 000 for women.[25] This paradoxical trend in mortality from ischemic heart disease was probably due to the improvement of other risk factors, such as a substantial reduction of blood pressure levels for both men and women, and a decline in the proportion of male smoking. In this study, meat consumption was inversely associated with blood pressure levels and smoking, suggesting that Japanese with moderate meat consumption had a beneficial profile of these cardiovascular risk factors.

The meta-analysis of 20 studies (1218 380 individuals at risk and 23 889 coronary heart diseases) showed that processed meat, but not red meat, was associated with higher risk of coronary heart disease.[7] The overall relative risks (95% CI) for coronary heart disease were 1.42 (1.07–1.89) per 50 g serving of processed meat per day and 1.00 (0.81–1.23) per 100 g serving of red meat per day.[7] However, as far as moderate meat consumption (up to 100 g/day), previous studies of Americans and Europeans revealed no excess risks of stroke,[8,9] ischemic heart disease[26] and total cardiovascular disease.[5]

We observed the potential excess risk of ischemic heart disease associated with meat consumption among overweight women (mean BMI = 27.2 kg/m^2). Our finding is constant with a cohort study of Americans women (BMI = 26 kg/m^2), which showed a significant association between meat consumption and risk of ischemic heart disease.[26]

We excluded deaths that occurred within 1–8 years from the baseline in order to reduce the potential bias of preexisting illness and disease (such as dyslipidemia, diabetes mellitus and hypertension), which may have lowered individual's meat consumptions due to a belief that meat may be harmful to their health. However, after the exclusion of early deaths within 8 years

Table 3. Sex-specific multivariable hazard ratios (HRs) and 95% confidence intervals (95% CIs) of mortality from ischemic heart disease according to quintiles of each meat consumptions (energy adjusted)

	Quintile of each meat consumptions					P *for trend*[a]
	Q1 (low) reference	*Q2 HR 95% CI*	*Q3 HR 95% CI*	*Q4 HR 95% CI*	*Q5 (high HR 95% CI*	
Men (*n*)	4093	4093	4094	4093	4093	
Red meat						
Median consumption (g/day)	6.4	15.5	23.3	33.2	57.8	
Person-years	62 785	64 247	64 694	64 366	62 995	
No. of cases	69	74	53	59	46	
Multivariable HR[b]	1.00	1.19 (0.85-1.65)	0.88 (0.61 - 1.27)	1.00 (0.70 - 1.44)	0.7 (0.47 - 1.04)	0.038
Poultry						
Median consumption (g/day)	1.9	3.3	10.2	13.3	27.3	
Person-years	63 758	62 452	65 983	64 031	62 863	
No. of cases	67	68	51	49	66	
Multivariable HR[b]	1.00	0.85 (0.58 - 1.25)	0.93 (0.63 - 1.37)	0.63 (0.41 - 0.96)	0.86 (0.60 - 1.23)	0.405
Processed meat						
Median consumption (g/day)	1.2	1.5	2.9	6.1	13.9	
Person-years	64 599	62 210	61 509	66 035	64 734	
No. of cases	62	72	74	50	43	
Multivariable HR[b]	1.00	1.01 (0.66 - 1.54)	0.87 (0.55 - 1.37)	0.80 (0.53 - 1.21)	0.56 (0.36 - 0.88)	0.002
Liver						
Median consumption (g/day)	1.2	1.5	2.7	3.6	15.2	
Person-years	66 472	62 899	64 653	63 822	61 240	
No. of cases	56	74	63	42	66	
Multivariable HR[b]	1.00	0.95 (0.61 - 1.48)	1.02 (0.69 - 1.51)	0.72 (0.46 - 1.15)	0.95 (0.63 - 1.42)	0.838
Women (*n*)	6243	6244	6243	6244	6243	
Red meat						
Median consumption (g/day)	4.0	11.5	17.8	25.5	43.9	
Person-years	989 01	100 792	101 160	100 591	99 544	
No. of cases	77	47	30	38	44	
Multivariable HR[b]	1.00	0.96 (0.66 - 1.40)	0.71 (0.46 - 1.10)	1.02 (0.68 - 1.54)	1.23 (0.82 - 1.85)	0.317
Poultry						
Median consumption (g/day)	1.5	4.2	8.6	11.3	22.4	
Person - years	99 439	98 542	102 799	99 074	101 134	
No. of cases	48	55	40	50	43	
Multivariable HR[b]	1.00	1.09 (0.72 - 1.66)	1.24 (0.78 - 1.98)	1.12 (0.72 - 1.74)	1.06 (0.69 - 1.62)	0.888
Processed meat						
Median consumption (g/day)	0.9	1.2	2.2	4.7	10.4	
Person-years	100 156	97 701	97896	103 346	101 890	
No. of cases	44	60	63	32	37	
Multivariable HR[b]	1.00	1.12 (0.68 - 1.84)	1.04 (0.61 - 1.84)	0.92 (0.56 - 1.50)	0.98 (0.59 - 1.62)	0.631
Liver						
Median consumption (g/day)	0.9	1.0	1.6	2.3	11.1	
Person-years	103 829	100 394	98 706	100 520	97 540	
No. of cases	42	54	55	31	54	
Multivariable HR[b]	1.00	0.91 (0.50 - 1.67)	0.66 (0.37 - 1.19)	0.88 (0.51 - 1.51)	1.01 (0.60 - 1.68)	0.166

[a]On the basis of the tests for trend across quintiles of meat consumption by assigning the median value of each quintile. [b]Cox proportional hazard models adjusted for the same variables shown in the footnote of Table 2.

of the baseline, the association did not change substantially, suggesting that the reverse causation is unlikely.

Several mechanisms were considered about adverse effects of meat consumption, because of high amount of heme iron, saturated fat and for processed meat, sodium. Heme iron increases risks of carotid atherosclerosis and type 2 diabetes, probably through the form action of hydroxyl radicals.[27,28] Oxidative stress induces insulin resistance through inhibition of normal phosphorylation of insulin receptor substrate proteins, and reduction of bioavailability of nitric oxide as a regulator of endothelial function through the peroxynitrite formation.[29] High sodium intake increases blood pressure levels.[30,31] High intake of saturated fat rich in meat increases total and low-density lipoprotein cholesterol levels.[32] Taken together, all of these effects may contribute to increase the risk of ischemic heart disease. However, in this study, total meat consumption was not associated with serum total cholesterol levels. Therefore, the positive association between total meat consumption and mortality from ischemic heart disease among the overweighted women was not explained by serum total cholesterol.

Table 4. Sex-specific multivariable hazard ratios (HRs) and 95% confidence intervals (95% CIs) of mortality from ischemic heart disease according to quintiles of total meat consumption stratified by body mass index (BMI)

	Quintile of total meat consumption					P *for trend*[a]
	Q1 (low)	*Q2*	*Q3*	*Q4*	*Q5 (high)*	
	reference	*HR 95% CI*	*HR 95% CI*	*HR 95% CI*	*HR 95% CI*	
Men						
BMI $< 25\,kg/m^2$						
Person-years	50 578	52 764	52 929	53 123	51 803	
No. of subjects	3339	3384	3360	3379	3389	
No. of cases	49	56	51	36	40	
Multivariable HR[b]	1.00	1.23 (0.83 - 1.82)	1.12 (0.75 - 1.68)	0.78 (0.50 - 1.21)	0.75 (0.48 - 1.18)	0.048
BMI $\geqslant 25\,kg/m^2$						
Person-years	12 082	11 543	11 832	11 282	11 151	
No. of subjects	754	709	734	714	704	
No. of cases	25	9	12	13	10	
Multivariable HR[b]	1.00	0.39 (0.18 - 0.86)	0.52 (0.25 - 1.09)	0.61 (0.30 - 1.25)	0.36 (0.15 - 0.83)	0.043
Women						
BMI $< 25\,kg/m^2$						
Person-years	76 124	78 615	79 825	80 296	79 673	
No. of subjects	4826	4905	4953	4990	4965	
No. of cases	56	35	33	29	25	
Multivariable HR[b]	1.00	0.99 (0.64 - 1.53)	1.07 (0.68 - 1.67)	0.98 (0.61 - 1.57)	0.87 (0.52 - 1.45)	0.623
BMI $\geqslant 25\,kg/m^2$						
Person-years	22 733	21 577	21 093	20 293	20 761	
No. of subjects	1417	1339	1290	1254	1278	
No. of cases	17	9	6	6	20	
Multivariable HR[b]	1.00	0.64 (0.27 - 1.47)	0.58 (0.22 - 1.53)	0.53 (0.20 - 1.40)	2.04 (0.98 - 4.26)	0.040

[a]On the basis of the tests for trend across quintiles of meat consumption by assigning the median value of each quintile. [b]Cox proportional hazard models adjusted for the same variables shown in the footnote of Table 2.

In addition, the mechanisms for the no positive association which observed between meat consumption and cardiovascular disease mortality observed in the present study are uncertain, but a potential effect of amino acids can be considered. Several amino acids such as branched-chain amino acids, L-arginine, tryptophan and tyrosine are richer in animal foods but poor in rice. Branched-chain amino acids promote the anabolic effect of cardiac protein, which may protect heart from ischemic damage.[33] L-arginine, a substrate for nitric oxide, produces an immediate reduction in systolic and diastolic blood pressures in humans.[34] Tryptophan and tyrosine have an antihypertensive effect in rats due to serotonin formation in the central nervous system.[35,36] However, we need further investigations whether these bioactivities affect risk of cardiovascular disease through moderate meat consumption.

The strengths of our study include its large cohort size, which provided us with strong statistical power, and a single study with a standardized protocol. The Japanese population's mean levels of total meat consumption were approximately half to one-third of those of Western people.[16] Traditional Japanese diet consists rice, miso soup and three dishes. One of the dishes is meat or fish, and the other two dishes are usually vegetables. In addition, the common cooking way of meat is boiling or stir-frying with vegetables rather than grilling like barbecue, the portion size of meat is generally small. Therefore, our study could effectively examine the relationship between moderate meat consumption and mortality from cardiovascular disease.

There were also several limitations in this study. First, for people who selected the highest category of meat consumption frequency (namely, almost daily), we could not estimate how many times they ate meat daily. This may have led to the under-estimation of meat consumption. The estimated median meat consumption in this study (33.7 g/day for men and 27.0 g/day for women) was less than half that estimated from dietary records in the validation study (75.9 g/day among men and 55.8 g/day among women).[19] Although the amount of meat consumed could be underestimated, the ranking of meat consumption was unlikely to change in this study. Second, a number of participants were excluded because they did not respond sufficiently to the FFQ. The non-respondents were older (58.8 years compared to 55.9 years), highly educated (high school or lower education achieved: 6% compared to 13%) and male (44 versus 40%), compared with respondents, although there were small differences in other baseline characteristics. Thus, a potential selection bias may be small. Finally, we cannot negate the possibility of residual confounding by other unexamined lifestyles or socio-economic status.

In conclusion, moderate meat consumption, up to $\sim$100 g/day, was not associated with increased mortality from ischemic heart disease, stroke or total cardiovascular disease among Japanese men and women. The potential excess risk of ischemic heart disease associated with meat consumption among overweight women needs further investigation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Office for Life-Style Related Diseases Control, General Affairs Division, Health Service Bureau, Ministry of Health, Labour and Welfare. *The National Health and Nutrition Survey in Japan. 2006*. Daiichi Shuppan: Tokyo, Japan 2009 (in Japanese).
2 Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. *JAMA* 2002; **288**, 2569 - 2578.
3 Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA *et al*. Dietary fat intake and the risk of coronary heart disease in women. *N Engl J Med* 1997; **337**, 1491 - 1499.
4 Ascherio A, Rimm EB, Giovannucci EL, Spiegelman D, Stampfer M, Willett WC. Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. *Br Med J* 1996; **313**, 84 - 90.
5 Sinha R, Cross AJ, Graubard BI, Leitzmann MF, Schatzkin A. Meat intake and mortality: a prospective study of over half a million people. *Arch Intern Med* 2009; **169**, 562 - 571.
6 Kelemen LE, Kushi LH, Jacobs Jr DR, Cerhan JR. Associations of dietary protein with disease and mortality in a prospective study of postmenopausal women. *Am J Epidemiol* 2005; **161**, 239 - 249.
7 Micha R, Wallace SK, Mozaffarian D. Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus: a systematic review and meta-analysis. *Circulation* 2010; **121**, 2271 - 2283.
8 Larsson SC, Virtamo J, Wolk A. Red meat consumption and risk of stroke in Swedish men. *Am J Clin Nutr* 2011; **94**, 417 - 421.
9 Larsson SC, Virtamo J, Wolk A. Red meat consumption and risk of stroke in Swedish women. *Stroke* 2011; **42**, 324 - 329.
10 Sauvaget C, Nagano J, Allen N, Grant EJ, Beral V. Intake of animal products and stroke mortality in the Hiroshima/Nagasaki Life Span Study. *Int J Epidemiol* 2003; **32**, 536 - 543.
11 Key TJ, Fraser GE, Thorogood M, Appleby PN, Beral V, Reeves G *et al*. Mortality in vegetarians and nonvegetarians: detailed findings from a collaborative analysis of 5 prospective studies. *Am J Clin Nutr* 1999; **70** (Suppl 3), S516 - S524.
12 Fraser GE. Associations between diet and cancer, ischemic heart disease, and all-cause mortality in non-Hispanic white California Seventh-day Adventists. *Am J Clin Nutr* 1999; **70** (Suppl 3), 532S - 538S.
13 Key TJ, Appleby PN, Spencer EA, Travis RC, Roddam AW, Allen NE. Mortality in British vegetarians: results from the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford). *Am J Clin Nutr* 2009; **89**, 1613S - 1619S.
14 Key TJ, Fraser GE, Thorogood M, Appleby PN, Beral V, Reeves G *et al*. Mortality in vegetarians and non-vegetarians: a collaborative analysis of 8300 deaths among 76000 men and women in five prospective studies. *Public Health Nutr* 1998; **1**, 33 - 41.
15 Appleby PN, Key TJ, Thorogood M, Burr ML, Mann J. Mortality in British vegetarians. *Public Health Nutr* 2002; **5**, 29 - 36.
16 Wang Y, Beydoun MA, Caballero B, Gary TL, Lawrence R. Trends and correlates in meat consumption patterns in the US adult population. *Public Health Nutr* 2010; **13**, 1333 - 1345.
17 Ohno Y, Tamakoshi A. Japan collaborative cohort study for evaluation of cancer risk sponsored by monbusho (JACC study). *J Epidemiol* 2001; **11**, 144 - 150.
18 Tamakoshi A, Yoshimura T, Inaba Y, Ito Y, Watanabe Y, Fukuda K *et al*. Profile of the JACC study. *J Epidemiol* 2005; **15** (Suppl 1), S4 - S8.
19 Date C, Fukui M, Yamamoto A, Wakai K, Ozeki A, Motohashi Y *et al*. Reproducibility and validity of a self-administered food frequency questionnaire used in the JACC study. *J Epidemiol* 2005; **15** (Suppl 1), S9 - S23.
20 Ministry of Health, Labour and Welfare. *The National Health and Nutrition Survey in Japan, 1990*. Daiichi Shuppan: Tokyo, Japan, 1992 (in Japanese).
21 Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997; **65** (Suppl 4), S1220 - S1231.
22 Iso H, Date C, Yamamoto A, Toyoshima H, Watanabe Y, Kikuchi S *et al*. Smoking cessation and mortality from cardiovascular disease among Japanese men and women: the JACC Study. *Am J Epidemiol* 2005; **161**, 170 - 179.
23 Iso H, Date C, Yamamoto A, Toyoshima H, Tanabe N, Kikuchi S *et al*. Perceived mental stress and mortality from cardiovascular disease among Japanese men and women: the Japan Collaborative Cohort Study for Evaluation of Cancer Risk Sponsored by Monbusho (JACC Study). *Circulation* 2002; **106**, 1229 - 1236.
24 Noda H, Iso H, Toyoshima H, Date C, Yamamoto A, Kikuchi S *et al*. Walking and sports participation and mortality from coronary heart disease and stroke. *J Am Coll Cardiol* 2005; **46**, 1761 - 1767.
25 Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labor and Welfare. *Vital Statistics of Japan, 2008* vol **1**., Health & Welfare Statistics Association: Tokyo, Japan, 2010.
26 Bernstein AM, Sun Q, Hu FB, Stampfer MJ, Manson JE, Willett WC. Major dietary protein sources and risk of coronary heart disease in women. *Circulation* 2010; **122**, 876 - 883.
27 Kiechl S, Willeit J, Egger G, Poewe W, Oberhollenzer F. Body iron stores and the risk of carotid atherosclerosis: prospective results from the Bruneck study. *Circulation* 1997; **96**, 3300 - 3307.
28 Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. *JAMA* 2004; **291**, 711 - 717.
29 Pitocco D, Zaccardi F, Di Stasio E, Romitelli F, Santini SA, Zuppi C *et al*. Oxidative stress, nitric oxide, and diabetes. *Rev Diabet Stud* 2010; **7**, 15 - 25.
30 Chamarthi B, Williams JS, Williams GH. A mechanism for salt-sensitive hypertension: abnormal dietary sodium-mediated vascular response to angiotensin-II. *J Hypertens* 2010; **28**, 1020 - 1026.
31 He FJ, Marciniak M, Visagie E, Markandu ND, Anand V, Dalton RN *et al*. Effect of modest salt reduction on blood pressure, urinary albumin, and pulse wave velocity in white, black, and Asian mild hypertensives. *Hypertension* 2009; **54**, 482 - 488.
32 Clarke R, Frost C, Collins R, Appleby P, Peto R. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. *Br Med J* 1997; **314**, 112 - 127.
33 Schwalb H, Kushnir T, Navon G, Yaroslavsky E, Borman JB, Uretzky G. The protective effect of enriched branched chain amino acid formulation in the ischemic heart: a phosphorous-31 nuclear magnetic resonance study. *J Mol Cell Cardiol* 1987; **19**, 991 - 998.
34 Hishikawa K, Nakaki T, Suzuki H, Kato R, Saruta T. Role of L-arginine-nitric oxide pathway in hypertension. *J Hypertens* 1993; **11**, 639 - 645.
35 Sved AF, Van Itallie CM, Fernstrom JD. Studies on the antihypertensive action of L-tryptophan. *J Pharmacol Exp Ther* 1982; **221**, 329 - 333.
36 Sved AF, Fernstrom JD, Wurtman RJ. Tyrosine administration reduces blood pressure and enhances brain norepinephrine release in spontaneously hypertensive rats. *Proc Natl Acad Sci USA* 1979; **76**, 3511 - 3514.

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