Relationship between Meat/Fish Consumption and Biliary Tract Cancer: The Japan Public Health Center–Based Prospective Study

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ABSTRACT

Background: The effect of meat and fish consumption on cancer risk has been well studied in humans. However, studies related to biliary tract cancer (BTC) are scarce.

Methods: We examined the association between meat and fish consumption and the risk of BTC in a population-based prospective cohort study in Japan. HRs and 95% confidence intervals (CI) were estimated using the Cox proportional hazard model.

Results: During 1995 and 1999, 43,177 men and 49,323 women ages 45 to 74 years were enrolled and followed up for 607,757.0 person-years (men) and 728,820.3 person-years (women) until 2012, during which time 217 male and 162 female BTC cases were identified. Higher total meat consumption was significantly associated with a decreased BTC risk in men (HR for the highest vs. lowest quartiles = 0.66; 95% CI, 0.44–0.98; $P_{trend} = 0.011$) but not in women. Similar association was observed with red meat, but no association was observed with poultry. Fish was not associated with BTC risk. We further analyzed each BTC subtype to confirm the observed association with BTC. However, significant association with each BTC subtype was not observed, although a trend of decreased extrahepatic bile duct cancer risk was observed.

Conclusions: BTC risk was lower among men who consumed more meat, particularly red meat, in Japan.

Impact: This is the first prospective study that evaluated the relationship between meat and BTC. This may provide important suggestions to elucidate the etiology of BTC.

Introduction

Dietary patterns are considered to be closely related to cancer risk, and the effects of meat and fish consumption on the risk of various types of cancer have been studied in humans. The World Cancer Research Fund/American Institute for Cancer Research reported that the evidence that consumption of red meat increases cancer risk is "probable" for colorectal cancer and "limited-suggestive" for lung, pancreas, and nasopharynx cancers; the evidence for processed meats is "convincing" in colorectal cancer, "probable" in stomach cancer, and "limited-suggestive" in esophagus, lung, stomach, pancreas, and nasopharynx cancers (dietandcancerreport.org). The potential mechanism of increased cancer risk for red meat and processed meats involves carcinogens such as heterocyclic amines, polycyclic aromatic hydrocarbons, N-nitroso compounds, heme iron, and production of secondary bile acids (dietandcancerreport.org; refs. 1–3). There has been inconsistent evidence regarding the influence of poultry consumption on cancer risk (dietandcancerreport.org; ref. 2). Fish has been well studied in liver and colorectal cancers, with "limited-suggestive" evidence that fish consumption decreases the risk of colorectal and liver cancers (dietandcancerreport.org).

Biliary tract cancer (BTC), including gallbladder cancer, extrahepatic bile duct cancer (EHBDC), and ampulla of Vater cancer (AVC) are highly fatal malignancies. Although BTC is globally rare, the incidence is more frequent in East Asia (4). In Japan, BTC was the 7th leading cause of cancer-related death in 2016 (https://ganjoho.jp/reg_stat/statistics/dl/index.html). Chronic inflammation of the biliary tract is strongly suggested to be related to the etiology of cancer in the biliary system (e.g., primary sclerosing cholangitis, pancreaticobiliary maljunction, cholelithiasis, hepatolithiasis, and dysplasia of gallbladder mucosa; refs. 5–8). Evidence of the association of meat and fish consumption with BTC risk is very limited. There have been several epidemiologic studies conducted, with inconsistent results regarding the association between BTC and consumption of both meat (9–13) and fish (9, 12, 14–16). Importantly, all the studies of meat consumption were retrospective studies; there have been no reports of findings from prospective studies.

We investigated the association between total meat and fish consumption and the risk of BTC in a population-based prospective cohort study in Japan. We further analyzed the association of total meat/fish with subtypes of BTC, as well as the association of each type of meat with BTC. In addition, intrahepatic bile duct cancer (IHBDC) was analyzed separately from BTC subtypes in this study because, although IHBDC is categorized as a type of liver cancer, the IHBD, and EHBD form one continuous structure.

Materials and Methods

Study cohort and participants

The Japan Public Health Center–based Prospective Study (JPHC Study) is a cohort study that mainly investigates noncommunicable

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Cancer Epidemiol Biomarkers Prev 2020;29:95–102

doi: 10.1158/1055-9965.EPI-19-0514

diseases. The JPHC study comprises two cohorts (cohort I started in 1990 and cohort II started in 1993), consisting of 140,420 participants from 11 public health centers (PHC), and both of the two cohorts were used for this study. The design of the study has been described in detail previously (17). The JPHC study was approved by the Institutional Review Board of the National Cancer Center (Tokyo, Japan). This study was approved by the Ethical Review Board of Osaka University (Osaka, Japan). We enrolled participants in this study who responded to a 5-year follow-up survey.

Out of total participants from two cohorts, 121,546 participants were initially identified as eligible after excluding participants due to the following reasons: the area where cancer incidence data were not collected ($n = 7{,}097$); non-Japanese nationality ($n = 51$); moving out of the study area before follow-up started ($n = 188$); incorrect birth date ($n = 7$); duplicate registration ($n = 10$); and death, moving out of a study area or loss to follow-up before the starting point in this study ($n = 11{,}521$). A total of 98,663 participants who were aged between 45 and 74 years responded to the 5-year follow-up survey from 1995 to 1999, yielding a response rate of 81.2%.

Exposure assessment

The self-administered questionnaire in the 5-year follow-up survey included a food frequency questionnaire (FFQ) that covered 138 food and beverage items with standard portions/units and nine frequency categories. Standard portion sizes were specified for all food items, with three amount choices: small (50% smaller than the standard), medium (the same as the standard), and large (50% larger than the standard) portions.

The FFQ asked about the average amount of consumption for 16 meat items and 19 fish and shellfish items during the previous year. These food items were categorized into the following groups: total meat (all meat items); red meat (all nonprocessed red meat items and all processed meat items); nonprocessed red meat [three beef items (steak, grilled beef, and stewed beef), six pork items (stir-fried pork, deep-fried pork, stewed pork in Western style, stewed pork in Japanese style, pork in soup, and pork liver), and chicken liver]; processed meat (four processed meat items: ham, sausage or Weiner sausage, bacon, and luncheon meat); poultry (two chicken items: grilled chicken and deep-fried chicken); and fish [all fish and shellfish items: salted fish, dried fish, canned tuna, salmon or trout, bonito or tuna, cod or flat fish, sea bream, horse mackerel or sardines, mackerel pike or mackerel, dried small fish, salted roe, eel, squid, octopus, prawn, short-necked clam, viviparidae, chikuwa (fish-paste product), and kamaboko (fish-paste product); refs. 18, 19]. Daily consumption of meat (g/day) was calculated by multiplying the frequency by the relative portion size. The validity of the FFQ for the assessment of meat consumption was evaluated using 14- or 28-day dietary records as the gold standard. For meat, the Spearman correlation coefficient (energy-adjusted) was 0.50 for men and 0.45 for women in cohort I, and 0.48 for men and 0.44 for women in cohort II (20, 21); for fish and shellfish, this was 0.32 for men and 0.32 for women in cohort I, and 0.27 for men and 0.23 for women in cohort II (20, 21). Reproducibility of the FFQ was evaluated by administering two questionnaires, 1 year apart. For meat, the Spearman correlation coefficient (energy-adjusted) was 0.52 for men and 0.52 for women in cohort I, and 0.52 for men and 0.41 for women in cohort II (21, 22); for fish and shellfish, this was 0.44 for men and 0.34 for women in cohort I, and 0.46 for men and 0.40 for women in cohort II (21, 22).

Follow-up and case identification

Residual status and survival information collected from the residential registers of each municipality in the study area was used for follow-up. Cause of death was confirmed using death certificates, with the permission of the Ministry of Health, Labor and Welfare (MHLW) of Japan. Death certificates were coded according to the requirements by the MHLW. Of the eligible participants, 5,366 moved outside the original area, 108 were lost to follow-up, 10 withdrew from the study, and 13,945 died (93.8% of participants completed the follow-up without moving outside the original area, being lost to follow-up, or withdrawal from the study).

Newly diagnosed cancer cases were determined by active patient notification from local major hospitals in the study area and data linkage with population-based cancer registries. Death certificate information was used as a supplementary information source. The site and histology of each cancer were coded in accordance with the International Classification of Diseases for Oncology, 3rd Edition, with the intrahepatic bile duct as C22.1, the gallbladder as C23.9, the extrahepatic bile duct as C24.0, ampulla of Vater as C24.1, overlapping sites of the biliary tract as C24.8, and unspecified as C24.9 in this analysis. If a patient was diagnosed with two or more of the aforementioned types of cancer, the cancer diagnosis with the earliest diagnosis date was used for this analysis. The proportion of cases for whom cancer diagnosis was ascertained by death certificate only was 10.5% for BTC and IHBDC and 6.5% for all types of cancer.

Statistical analyses

We further excluded the following participants out of the 98,663 participants: participants who had a history of cancer ($n = 3{,}879$); participants for whom the date at the end of follow-up was unknown ($n = 211$); and participants for whom reported dietary intake was unknown or extreme total energy intake (<500 or >5,000 kcal total energy) was reported ($n = 2{,}073$). Finally, 92,500 participants were included in the analysis.

The person-years of follow-up was counted for individual participants from the 5-year follow-up survey date to the end of follow-up, which was the earliest date out of the following events: moving outside the study area, loss to follow-up, withdrawal from the study, death, diagnosis of BTC and IHBDC, or the last date of the follow-up period (December 31, 2012). Participants lost to follow-up were censored at the last confirmed date of their presence in the study area.

The Cox proportional hazards model was used to estimate the HRs, 95% confidence intervals (CI), and P_{trends} for men and women with adjusting for potential confounders. We applied the residual model for energy adjustment of meat and fish consumption (23) and categorized the energy-adjusted consumption volume into tertiles or quartiles, with the lowest consumption category as the reference. P_{trend} was calculated by assigning ordinal values to each category and entering them as continuous terms in each model. All P values reported are two-sided and <0.05 was regarded as statistically significant. Stata version 13 (StataCorp LLC) was used to perform all statistical analysis.

The primary analysis was conducted to evaluate the association of total meat and fish consumption with BTC. Furthermore, we conducted the following two subanalyses: association of meat by subgroups [red meat (further divided into unprocessed red meat and processed meat) and poultry] with BTC, and association of total meat with BTC subtypes (gallbladder cancer and EHBDC) and IHBDC. BTC was analyzed using quartile categories of food consumption, and BTC subtypes and IHBDC were analyzed using tertile categories because of the limited number of cases. This multivariable analysis model was adjusted for the following potential confounding variables that were biologically *a priori* and/or were considered associated with general cancer risk or BTC risk (dietandcancerreport.org;

refs. 7, 24–27): age (continuous); study area (10 PHC areas); body mass index (BMI; <23, 23–25, 25–27, and $\geq$27 kg/m^2); history of cholelithiasis (no/yes); history of diabetes mellitus (no/yes); history of chronic hepatitis or cirrhosis (no/yes); history of smoking (no, past or current, and unknown); alcohol consumption frequency (never or almost never, 1–3 times/month, 1–2 times/week, 3–4 times/week, $\geq$5 times/week, and unknown); physical activity by metabolic equivalents/day (quartiles and unknown); green tea consumption ($\leq$120, 120–360, 360–720, or >720 mL/day, and unknown); and energy-adjusted consumption of fish [quartiles (only for meat analysis)], total meat [quartile (only for fish analysis)], and vegetables + fruits (quartiles). For the analysis of meat subgroups, the model was further adjusted for the other meat groups. Furthermore, we excluded participants diagnosed during the first 3 years of the follow-up period and performed the same analysis as above. We tested proportional hazard assumption using Shenfield residuals, and they were not statistically significant for each of total meat and fish consumption in men, but were significant for total meat consumption in women. Therefore, we show the women data for meat consumption just as a reference in this article. The survival curves for BTC incidence by total meat and fish consumption in men and women are shown in Supplementary Fig. S1.

We referred to previous studies that investigated BTC when developing the study methods (26–29).

Results

Baseline characteristics of the participants are shown in **Table 1A** (by total meat consumption) and **Table 1B** (by fish consumption). A total of 43,177 men and 49,323 women were included and followed-up for 607,757.0 person-years in men and 728,820.3 person-years in women. During the follow-up period, 217 cases of BTC (65 gallbladder cancer, 119 EHBDC, 25 AVC, and 8 unspecified) and 71 IHBDC cases were identified in men; and 162 BTC cases (84 gallbladder cancer, 56 EHBDC, 13 AVC, and 9 unspecified) and 55 IHBDC cases were identified in women. Men with higher meat consumption tended to be younger and consumed less vegetables + fruits, and were less likely have a history of diabetes mellitus or liver disease. Women with higher meat consumption tended to be younger, consume less vegetables + fruits, and were less likely to have a history of diabetes mellitus or liver disease. Men with higher fish consumption tended to be older and consume more vegetables + fruits and green tea; they were more likely have a history of diabetes mellitus or liver disease. Women with higher fish consumption tended to be older and consume more vegetables + fruits and green tea.

The HRs and 95% CIs of BTC incidence according to quartiles of total meat and fish consumption in men and women are shown in **Table 2**. Increased total meat consumption was significantly associated with decreased risk of BTC in men [HR = 0.66 (95% CI, 0.44–0.98) for the highest group; $P_{\text{trend}} = 0.011$], while it was not significantly associated with BTC risk in women. Fish consumption was not significantly associated with BTC risk in either men or women.

The HRs and 95% CIs of BTC incidence according to quartiles of meat consumption by subtype in men and women are shown in **Table 3**. In men, increased consumption of red meat was significantly associated with decreased risk of BTC [HR = 0.59 (95% CI, 0.38–0.90) for the highest consumption group; $P_{\text{trend}} = 0.005$), while increased consumption of poultry was not associated with BTC risk. In the analysis after further dividing red meat into unprocessed red meat and processed meat, a trend of decreased BTC risk associated with increased consumption was observed for both unprocessed red meat and processed meat. In women, increased consumption of poultry and red meat was not associated with BTC risk.

HRs and 95% CIs of BTC subtypes incidence according to tertiles of total meat and fish consumption in men and women are shown in **Table 4**. In men, increased total meat consumption was not significantly associated with gallbladder cancer or EHBDC, although we observed a nonsignificant trend of decreased risk of EHBDC [HR = 0.67 (95% CI, 0.42–1.07) for the highest consumption group; $P_{\text{trend}} = 0.069$). No significant association was observed with total meat consumption in women and with fish consumption in either men or women.

Results from the analysis after excluding participants diagnosed within the first 3 years of the follow-up period were similar (Supplementary Tables S1–S3).

Discussion

In this population-based prospective cohort study in Japan, we investigated the association of meat and fish consumption with the risk of BTC (including gallbladder cancer, EHBDC, and AVC) and IHBDC in both men and women. To our knowledge, there has not been any reported result from prospective study about the association of meat consumption with BTC. High total meat consumption was significantly associated with decreased risk of BTC in men, while we did not observe a significant association between meat consumption and BTC risk in women. We further analyzed BTC subtypes as well as IHBDC to confirm the observed association of BTC in men. However, we did not observe significant association between each of BTC subtype and total meat consumption, although a nonsignificant trend of decreased risk and EHBDC was observed. Fish was not associated with BTC risk. In the analysis by subtype of meat, high consumption of red meat was significantly associated with decreased risk of BTC, and no association with poultry was observed in men.

The effect of meat on BTC risk suggested from the previous studies was unclear. Only a few studies have been reported, and all of these were retrospective studies. No significant association was observed in the study in Italy investigating the association between gallbladder cancer ($n = 60$) and red meat (10), the study in Thailand investigating the association of cholangiocarcinoma ($n = 108$) with red meat and poultry in Thailand (11), the study in India investigating the association between gallbladder cancer ($n = 64$) and beef, mutton, and poultry (12), and another study in Italy investigating the association between processed meat and biliary tract cancer ($n = 159$; ref. 13). On the other hand, one study conducted in Japan showed a significantly decreased risk of gallbladder cancer ($n = 109$) associated with total of pork, beef, and chicken consumption (9).

In this study, total meat and red meat consumption were inversely associated with the risk of BTC, and poultry consumption was not associated with BTC risk in men. The reasons and mechanisms for the observed association are unclear, but difference of subject background among groups by meat consumption and potentially preventive effect of nutrients contained in meat are considered as possible reasons. Regarding subject background difference, one of the previous studies reported that increased deprivation was associated with BTC risk. (30) Information about socioeconomic status (SES) is limited in this study, but the limited information suggested that men with higher academic background or professional occupation tended to be slightly more in the group of high red meat consumption. Some factors that may be affected by SES such as smoking and drinking were adjusted in this study, but the possibility

Table 1A. Baseline characteristics of the study participants by quartiles of total meat consumption.

	Men				Women			
Quartiles (Median daily consumption of total meat)	**1Q (20.3)**	**2Q (42.5)**	**3Q (64.8)**	**4Q (104.9)**	**1Q (17.4)**	**2Q (38.0)**	**3Q (58.0)**	**4Q (92.9)**
Number of subjects	10,795	10,794	10,794	10,794	12,331	12,331	12,331	12,330
Person-years	149,880.0	152,335.4	153,347.2	152,194.4	180,400.9	182,301.1	182,356.5	183,761.8
Age (years), mean (SD)	58.3 (7.8)	57.0 (7.8)	56.2 (7.8)	56.2 (7.8)	58.8 (7.9)	57.3 (7.9)	56.6 (7.9)	56.4 (8.0)
BMI (kg/m^2), mean (SD)	23.4 (2.9)	23.5 (2.8)	23.6 (2.8)	23.8 (3.0)	23.4 (3.2)	23.4 (3.1)	23.4 (3.1)	23.7 (3.3)
History of cholelithiasis (yes), %	3.3	3.8	4.0	3.8	4.4	3.9	3.8	3.9
History of diabetes mellitus (yes), %	9.7	8.7	8.8	8.2	4.8	4.3	4.1	3.9
History of liver disease (%)	3.7	2.8	2.9	2.5	1.6	1.3	1.3	0.9
Current smoker (%)	42.5	45.3	45.3	43.0	5.1	4.8	5.2	5.9
Regular drinker ($\geq$1/week), %	68.5	69.8	67.4	58.7	11.4	13.8	13.2	11.1
Green tea intake (>120 mL/day), %	73.8	74.4	73.4	66.2	78.0	79.4	78.6	72.0
Physical activity (mean MET-hours/day)	33.9	33.7	33.6	33.4	32.7	32.7	32.8	32.6
Mean dietary intake								
Total energy (kcal)	2,193.0	2,196.4	2,157.4	2,106.9	1,905.1	1,876.9	1,860.7	1,842.9
Fish (g)	81.6	87.1	90.9	89.1	80.3	84.3	86.3	82.7
Total meat (g)	—	—	—	—	—	—	—	—
Vegetable + fruit (g)	361.3	370.8	361.4	331.8	494.9	483.6	449.1	386.5
Red meat (g)	15.3	35.3	55.1	100.2	13.0	31.4	49.2	89.0
Processed meat (g)	2.1	4.6	7.0	11.5	2.1	4.6	6.9	10.8
Poultry (g)	3.5	7.3	10.3	15.6	3.1	6.7	9.4	14.2

Abbreviation: MET, metabolic equivalents.

that unadjusted residual by SES may affect the association between red meat consumption and BTC risk cannot be ruled out. Another possibility of potential impact by subject background difference is disease history. There seemed a trend that the proportion of men with a history of diabetes mellitus or liver disease was lower in the group of higher meat consumption as shown in **Table 1A**. Although the measured factors about disease history were adjusted in the multivariable model, unmeasured factors may affect the investigated association in this study. One potential mechanism is the preventive effect of monounsaturated fatty acids (MUFA). Red meat contains an abundance of MUFAs; in this study, the correlation between consumption of MUFAs and consumption of red meat was high ($r = 0.72$) and the correlation between MUFA and poultry consumption was moderate ($r = 0.33$) in men. The association between MUFA and BTC risk was explanatorily assessed, and a trend of decreased BTC and EHBDC risk, although it was not statistically significant,

Table 1B. Baseline characteristics of the study participants by quartiles of fish consumption.

	Men				Women			
Quartiles (Median daily consumption of fish)	**1Q (35.7)**	**2Q (63.9)**	**3Q (92.6)**	**4Q (143.8)**	**1Q (35.1)**	**2Q (62.7)**	**3Q (89.5)**	**4Q (135.1)**
Number of subjects	10,795	10,794	10,794	10,794	12,331	12,331	12,331	12,330
Person-years	152,440.0	152,271.7	152,591.5	150,453.8	180,610.1	181,752.9	182,696.3	183,761.1
Age (years), mean (SD)	56.3 (8.0)	56.3 (7.9)	56.8 (7.7)	58.2 (7.7)	57.4 (8.4)	56.6 (8.0)	57.1 (7.9)	58.0 (7.6)
BMI (kg/m^2), mean (SD)	23.7 (3.0)	23.6 (2.9)	23.5 (2.8)	23.5 (2.9)	23.6 (3.3)	23.4 (3.2)	23.4 (3.1)	23.6 (3.2)
History of cholelithiasis (yes), %	3.8	3.7	3.6	3.8	4.3	4.1	3.8	3.8
History of diabetes mellitus (yes), %	7.3	8.1	8.8	11.2	4.2	3.9	4.3	4.8
History of liver disease (%)	2.8	2.9	3.0	3.3	1.2	1.2	1.3	1.3
Current smoker (%)	43.6	45.0	44.8	42.7	5.7	5.2	4.9	5.0
Regular drinker ($\geq$1/week), %	65.1	67.3	67.9	64.1	10.9	12.7	13.5	12.4
Green tea intake (>120 mL/day), %	64.8	71.4	74.8	76.4	70.5	76.6	80.9	79.8
Physical activity (mean MET-hours/day)	33.8	33.6	33.6	33.6	32.6	32.7	32.8	32.6
Mean dietary intake								
Total energy (kcal)	2,202.7	2,168.5	2,162.5	2,120.0	1,899.3	1,863.4	1,873.9	1,848.9
Fish (g)	—	—	—	—	—	—	—	—
Total meat (g)	55.9	61.5	63.1	61.9	52.1	55.1	55.1	53.3
Vegetable + fruit (g)	303.4	351.4	374.6	395.8	410.2	456.7	474.2	473.0
Red meat (g)	49.2	52.4	53.0	51.3	45.7	47.0	46.0	43.8
Processed meat (g)	5.8	6.6	6.5	6.2	5.8	6.3	6.4	5.9
Poultry (g)	6.8	9.1	10.2	10.6	6.5	8.2	9.2	9.5

Abbreviation: MET, metabolic equivalents.

Table 2. HRs and 95% CIs of incidence of bile tract cancer, according to quartiles of total meat and fish consumption in men and women.

	Person-year	Cases	IR per 100,000	Age-adjusted HR (95% CI)	Multivariable-adjusted HR (95% CI)
Men					
Total meat					
First	149,880	74	49.4	1.00	1.00[a]
Second	152,335	60	39.4	0.87 (0.62–1.23)	0.88 (0.62–1.24)
Third	153,347	41	26.7	0.63 (0.43–0.92)	0.62 (0.42–0.92)
Fourth	152,194	42	27.6	0.65 (0.45–0.95)	0.66 (0.44–0.98)
P_{trend}				0.007	0.011
Per SD increase				0.83 (0.72–0.97)	0.84 (0.72–0.98)
Fish					
First	152,440	45	29.5	1.00	1.00[b]
Second	152,272	55	36.1	1.22 (0.82–1.81)	1.31 (0.88–1.97)
Third	152,591	52	34.1	1.12 (0.75–1.66)	1.25 (0.82–1.90)
Fourth	150,454	65	43.2	1.27 (0.87–1.86)	1.39 (0.92–2.08)
P_{trend}				0.309	0.173
Per SD increase				1.09 (0.97–1.23)	1.10 (0.98–1.23)
Women					
Total meat					
First	180,401	49	27.2	1.00	1.00[a]
Second	182,301	33	18.1	0.76 (0.49–1.17)	0.79 (0.51–1.24)
Third	182,356	46	25.2	1.12 (0.74–1.67)	1.18 (0.78–1.79)
Fourth	183,762	34	18.5	0.82 (0.53–1.28)	0.85 (0.54–1.35)
P_{trend}				0.777	0.933
Per SD increase				0.92 (0.78–1.08)	0.93 (0.79–1.10)
Fish					
First	180,610	46	25.5	1.00	1.00[b]
Second	181,753	35	19.3	0.82 (0.53–1.27)	0.80 (0.51–1.26)
Third	182,696	29	15.9	0.65 (0.41–1.04)	0.63 (0.39–1.03)
Fourth	183,761	52	28.3	1.09 (0.73–1.62)	1.04 (0.68–1.60)
P_{trend}				0.830	0.926
Per SD increase				1.07 (0.93–1.22)	1.06 (0.92–1.22)

Abbreviation: IR, incidence rate.
[a]Adjusted for age, area, BMI, history of cholelithiasis, history of diabetes mellitus, history of chronic hepatitis or cirrhosis, history of smoking, drinking frequency, physical activity, green tea consumption, fish consumption, and consumption of vegetables and fruits.
[b]Adjusted for age, area, BMI, history of cholelithiasis, history of diabetes mellitus, history of chronic hepatitis or cirrhosis, history of smoking, drinking frequency, physical activity, green tea consumption, total meat consumption, and consumption of vegetables and fruits.

was observed in moderate and high MUFA consumption groups as compared with low consumption group (Supplementary Table S4). Previous studies have investigated the association between cancer risk and consumption of olive oil, which contains abundant oleic acid (the predominant type of MUFA); study findings have suggested a protective effect on some types of cancers, such as breast cancer (31, 32). Oleic acid or the antioxidant components in olive oil are considered to contribute to this observed protective effect, although main contributors are unclear (33). The biological effects of oleic acid in preventing cancer include protection from oxidative DNA damage, modification of cell membrane structure and function, modulation of signal transduction pathways, modulation of genes involved in cell proliferation, and anti-inflammatory and immunomodulatory effects (34). Another potential mechanism is the effect of the micronutrients contained in meat. Meat is the major source for some micronutrients because of either high or better bioavailability such as folate, selenium, and zinc that may be cancer preventive (35). In addition, we assessed potential impact of cooking method difference (i.e., grilled/barbecued red meat, pan-fried red meat, fried red meat, and boiled red meat and liver) on the result of this study. However, no remarkable difference of proportion of kinds of meat by each cooking method among quartiles of total meat was observed in men and women.

The volume of red meat consumption in Japan may affect the inverse association observed in this study. During the 1990s, when the baseline survey in this study was conducted, per capita consumption of beef in Japan was less than half that in United States, and Japan's pork consumption was slightly more than half the U.S. levels (36). Previous researches in western countries demonstrated increased risk of cancer-related mortality associated with red meat consumption (37, 38), while pooled analysis of Asian cohort studies, which have included Japan, showed that red meat consumption is not associated with cancer-related mortality in men and was inversely associated with cancer-related mortality in women (36). As discussed above, red meat contains some cancer preventive as well as cancer-promoting components, so the effect of the protective components might predominate in the lower range of consumption volume. Moreover, the observed inverse association in this study may be specific to the study area, where the volume of meat consumption is low. Among previous studies on the association between meat consumption and BTC, the only study from Japan showed an inverse association with gallbladder cancer and the remaining studies reported no association (9–13). Further research in regions with both high and low levels of meat consumption is required to clarify this association.

In this study, total meat consumption was significantly associated with decreased BTC risk in men but not in women. The reasons for this

Table 3. HRs and 95% CIs of incidence of BTC, according to quartiles of poultry and red meat consumption stratified by sex.

	Men			Women		
Variable	IR per 100,000	Age-adjusted HR (95% CI)	Multivariable-adjusted HR (95% CI)	IR per 100,000	Age-adjusted HR (95% CI)	Multivariable-adjusted HR (95% CI)
Poultry						
First	42.4	1.00	1.00[a]	30.7	1.00	1.00[a]
Second	34.1	0.88 (0.61–1.28)	0.96 (0.66–1.39)	19.7	0.72 (0.47–1.09)	0.73 (0.47–1.13)
Third	35.1	0.96 (0.67–1.39)	1.12 (0.76–1.66)	15.8	0.62 (0.39–0.97)	0.64 (0.40–1.04)
Fourth	31.4	0.86 (0.59–1.25)	1.07 (0.71–1.61)	22.9	0.89 (0.60–1.34)	0.93 (0.59–1.45)
P_{trend}		0.522	0.590		0.415	0.610
Red meat						
First	51.4	1.00	1.00[b]	26.6	1.00	1.00[b]
Second	37.3	0.80 (0.57–1.13)	0.80 (0.56–1.13)	20.3	0.87 (0.57–1.34)	0.98 (0.63–1.52)
Third	27.4	0.62 (0.42–0.90)	0.60 (0.40–0.89)	23.6	1.07 (0.71–1.62)	1.19 (0.76–1.85)
Fourth	27.0	0.61 (0.42–0.89)	0.59 (0.38–0.90)	18.5	0.84 (0.54–1.30)	0.91 (0.55–1.49)
P_{trend}		0.003	0.005		0.665	0.953
Unprocessed red meat						
First	50.6	1.00	1.00[c]	26.0	1.00	1.00[c]
Second	35.3	0.78 (0.55–1.10)	0.81 (0.56–1.16)	19.7	0.85 (0.55–1.32)	0.95 (0.60–1.49)
Third	28.8	0.65 (0.45–0.94)	0.68 (0.45–1.01)	23.1	1.04 (0.69–1.58)	1.16 (0.74–1.82)
Fourth	28.3	0.63 (0.43–0.92)	0.66 (0.44–1.01)	20.2	0.89 (0.58–1.37)	0.96 (0.59–1.55)
P_{trend}		0.007	0.034		0.809	0.924
Processed meat						
First	54.3	1.00	1.00[d]	26.7	1.00	1.00[d]
Second	29.5	0.64 (0.44–0.92)	0.66 (0.45–0.96)	24.8	1.12 (0.74–1.68)	1.22 (0.80–1.86)
Third	36.0	0.82 (0.58–1.16)	0.87 (0.60–1.25)	18.1	0.91 (0.58–1.43)	1.00 (0.62–1.60)
Fourth	23.8	0.59 (0.40–0.88)	0.64 (0.41–0.98)	19.4	1.08 (0.70–1.68)	1.18 (0.72–1.91)
P_{trend}		0.026	0.096		0.953	0.703

Abbreviation: IR, incidence rate.

[a]Adjusted for age, area, BMI, history of cholelithiasis, history of diabetes mellitus, history of chronic hepatitis or cirrhosis, history of smoking, drinking frequency, physical activity, green tea consumption, fish consumption, vegetable and fruit consumption, and red meat consumption.

[b]Adjusted for age, area, BMI, history of cholelithiasis, history of diabetes mellitus, history of chronic hepatitis or cirrhosis, history of smoking, drinking frequency, physical activity, green tea consumption, fish consumption, vegetable and fruit consumption, and poultry consumption.

[c]Adjusted for age, area, BMI, history of cholelithiasis, history of diabetes mellitus, history of chronic hepatitis or cirrhosis, history of smoking, drinking frequency, physical activity, green tea consumption, fish consumption, vegetable and fruit consumption, poultry consumption, and processed meat consumption.

[d]Adjusted for age, area, BMI, history of cholelithiasis, history of diabetes mellitus, history of chronic hepatitis or cirrhosis, history of smoking, drinking frequency, physical activity, green tea consumption, fish consumption, vegetable and fruit consumption, poultry consumption, and unprocessed red meat consumption.

difference are unclear, but multiple factors such as volume of meat consumption (median volume is ~10% less in women), the number of BTC cases (~25% less in women), and proportion of BTC subtypes might contributed to this difference. Regarding BTC subtypes, a trend of decreased risk of EHBDC was observed in men, and EHBDC is the most dominant subtype [119 EHBDC cases of 217 BTC cases (54.8%)]. On the other hand, the number of EHBDC in women is approximately half of that in men and less dominant among BTC subtypes [56 EHBDC cases out of 162 BTC cases (34.6%)].

This study demonstrated a nonsignificant trend of decreased risk of EHBDC and IHBDC, respectively, associated with total meat consumption (**Table 4**; Supplementary Table S5). To our knowledge, this is the first study that specifically investigated IHBDC. One previous study investigated a risk of cholangiocarcinoma including both intrahepatic and extrahepatic cholangiocarcinoma associated with red meat and poultry, and no significant association was observed (11). The limitation for both studies is small number of cases due to low incidence. Further studies with larger sample size are required to evaluate the association of IHBDC risk with meat consumption.

Fish consumption was not significantly associated with BTC risk in men and women although slightly increased risk was observed in men. The results from the previous studies were inconsistent because two studies showed decreased risk (9, 16), no association was observed in one study (12), and increased risk was observed in another study (14, 15). Further study is required to confirm the association between fish consumption and BTC risk.

This study has several limitations. First, the statistical power for the analysis, especially by each BTC subtype, was not sufficient because of the low incidence rates. As heterogeneity among these subtypes has been suggested (39, 40) and no statistically significant association with any subtype was observed in this study, the possibility that the observed inverse association of meat consumption with BTC was owing to chance cannot be ruled out. Therefore, studies with larger sample sizes for each subtype would be required to confirm the results of this study. Second, our study findings may not be generalizable to other countries where the amount of meat consumption differs from that in Japan. Third, there could have been some misclassification of exposure categories because exposure was assessed only at a single point. In addition, the moderate correlation coefficient of meat might mask the true association. Fourth, there could be some effects owing to unmeasured variables of lifestyle factors and disease history associated with meat consumption and residual confounding.

In this study population, consumption of meat, particularly red meat, was significantly associated with decreased risk of BTC in men,

Table 4. HRs and 95% CIs of BTC subtypes (gallbladder cancer and EHBDC) according to tertiles of total meat and fish consumption stratified by sex.

	Men			Women		
Variable	IR per 100,000	Age-adjusted HR (95% CI)	Multivariable-adjusted HR (95% CI)	IR per 100,000	Age-adjusted HR (95% CI)	Multivariable-adjusted HR (95% CI)
Total meat						
GBC						
First	15.4	1.00	1.00[a]	12.4	1.00	1.00[a]
Second	4.4	0.32 (0.15–0.68)	0.31 (0.15–0.66)	11.1	1.02 (0.60–1.71)	1.06 (0.62–1.79)
Third	12.3	0.93 (0.55–1.57)	0.80 (0.46–1.39)	11.0	1.04 (0.62–1.76)	1.05 (0.61–1.82)
P_{trend}		0.665	0.352		0.873	0.844
EHBDC						
First	28.4	1.00	1.00[a]	10.4	1.00	1.00[a]
Second	16.7	0.65 (0.42–0.99)	0.71 (0.46–1.09)	6.6	0.73 (0.39–1.37)	0.77 (0.41–1.46)
Third	13.8	0.55 (0.35–0.86)	0.67 (0.42–1.07)	6.1	0.70 (0.37–1.33)	0.73 (0.38–1.42)
P_{trend}		0.006	0.069		0.251	0.331
Fish						
GBC						
First	10.3	1.00	1.00[b]	13.7	1.00	1.00[b]
Second	9.3	0.89 (0.48–1.65)	1.05 (0.55–2.00)	8.6	0.67 (0.39–1.15)	0.62 (0.35–1.10)
Third	12.4	1.05 (0.59–1.88)	1.33 (0.72–2.47)	12.3	0.88 (0.54–1.44)	0.80 (0.47–1.35)
P_{trend}		0.841	0.359		0.604	0.430
EHBDC						
First	15.7	1.00	1.00[b]	8.3	1.00	1.00[b]
Second	16.7	1.04 (0.64–1.69)	1.08 (0.66–1.77)	4.9	0.63 (0.31–1.29)	0.63 (0.30–1.33)
Third	26.4	1.50 (0.97–2.33)	1.47 (0.93–2.34)	9.8	1.15 (0.63–2.08)	1.12 (0.60–2.11)
P_{trend}		0.057	0.086		0.609	0.629

Abbreviations: GBC, gallbladder cancer; IR, incidence rate.
[a]Adjusted for age, area, BMI, history of cholelithiasis, history of diabetes mellitus, history of chronic hepatitis or cirrhosis, history of smoking, drinking frequency, physical activity, green tea consumption, fish consumption, and vegetable and fruit consumption.
[b]Adjusted for age, area, BMI, history of cholelithiasis, history of diabetes mellitus, history of chronic hepatitis or cirrhosis, history of smoking, drinking frequency, physical activity, green tea consumption, total meat consumption, and vegetable and fruit consumption.

while no significant association was observed in women. However, the observed inverse association is considered inconclusive so far because, in the analysis of BTC subtypes, total meat consumption was not significantly associated with any subtype, although a nonsignificant trend of decreased EHBDC risk was observed. These results warrant further study, to confirm the observed association of meat and elucidate the underlying reasons or mechanisms.

Disclosure of Potential Conflicts of Interest

T. Makiuchi is Medical Affairs Lead, Senior Manager at Eli Lilly Japan K.K. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

The authors are indebted to the Aomori, Iwate, Ibaraki, Niigata, Osaka, Kochi, Nagasaki, and Okinawa Cancer Registries for providing their incidence data. This article was supported by the Clinical Investigator's Research Project in Osaka University Graduate School of Medicine. This study was supported by the National Cancer Center Research and Development Fund (23-A-31[toku] and 26-A-2; since 2011), a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan (from 1989 to 2010), and a grant from commissioned project study, Ministry of Agriculture, Fishery and Forestry, Japan (MAFF-CPS-2016-1-1). JPHC members are listed at the following site (as of April 2017): http://epi.ncc.go.jp/en/jphc/781/7951.html.

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Received May 11, 2019; revised July 31, 2019; accepted October 15, 2019; published first October 22, 2019.

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