Meat consumption and cancer of unknown primary (CUP) risk: results from The Netherlands cohort study on diet and cancer

Karlijn E. P. E. Hermans1, Piet A. van den Brandt1, Caroline Loef2, Rob L. H. Jansen3, Leo J. Schouten1

Received: 29 January 2021 / Accepted: 26 May 2021 / Published online: 21 June 2021
© The Author(s) 2021

Abstract
Purpose Cancer of unknown primary (CUP) is a metastasised cancer for which no primary lesion could be identified during life. Research into CUP aetiology with respect to dietary factors is particularly scarce. This study investigates whether meat consumption is associated with CUP risk.

Methods Data was utilised from the prospective Netherlands cohort study that includes 1,20,852 participants aged 55–69 years. All participants completed a self-administered questionnaire on diet and other cancer risk factors at baseline. Cancer follow-up was established through record linkage to the Netherlands Cancer Registry and the Dutch Pathology Registry. A total of 899 CUP cases and 4111 subcohort members with complete and consistent dietary data were available for case–cohort analyses after 20.3 years of follow-up. Multivariable adjusted hazard ratios (HRs) were calculated using proportional hazards models.

Results We found a statistically significant positive association with beef and processed meat consumption and CUP risk in women (multivariable adjusted HR Q4 vs. Q1 1.47, 95% CI 1.04–2.07, \(P_{\text{trend}} = 0.004\) and Q4 vs. Q1 1.53, 95% CI 1.08–2.16, \(P_{\text{trend}} = 0.001\), respectively), and a non-significant positive association with processed meat consumption and CUP risk in men (multivariable adjusted HR Q4 vs. Q1 1.33, 95% CI 0.99–1.79, \(P_{\text{trend}} = 0.15\)). No associations were observed between red meat (overall), poultry or fish consumption and CUP risk.

Conclusion In this cohort, beef and processed meat consumption were positively associated with increased CUP risk in women, whereas a non-significant positive association was observed between processed meat consumption and CUP risk in men.

Keywords Cancer of unknown primary (CUP) · Red meat · Processed meat · Poultry · Fish · Prospective cohort study

Abbreviations
CI Confidence interval
CUP Cancer of unknown primary
HR Hazard ratio
NCR Netherlands cancer registry
NLCS Netherlands cohort study on diet and cancer
PALGA Dutch pathology registry

Introduction
Cancer of unknown primary (CUP) is a metastasised malignancy for which the primary tumour origin remains unidentifiable during a patient’s lifetime [1, 2]. It ranks fourth in the most common metastasised cancers in the Netherlands, and with slightly more than 1300 incident cases in 2018, CUP accounted for almost 2% of all new cancer diagnoses in that year [3, 4]. Globally, the median survival for CUP patients is only 3 months, dependent on available diagnostics as well as incidence and patient characteristics (favourable or unfavourable prognosis, 20–80%, respectively) [5–7]. For most CUP patients, curative treatment(s) may no longer be an option [8]. By assessing lifestyle factors that are potentially
been linked to the development of CUP [9–12]. Nonetheless, the relationship between diet and CUP has been less well studied, and that is particularly true with respect to meat consumption [11].

The consumption of red meat and processed meat has been linked to several types of cancer in previous studies [13]. Indeed, the weight of evidence is such that the International Agency for Research on Cancer (IARC) describes red meats as “probably carcinogenic to humans”, and there is also sufficient evidence to classify processed meats as “carcinogenic to humans” [13]. Red meats are unprocessed mammalian muscle meat that contain proteins and important micronutrients, such as B vitamins, iron, and zinc [13, 14]. Processed meats, by contrast, are those meats that have been transformed through salting, curing, fermentation, smoking, or other processes so as to enhance their flavour or improve their preservation [13]. When those meats are being processed, it can lead to the formation of carcinogenic chemicals (including N-nitroso-compounds (NOC) and polycyclic aromatic hydrocarbons (PAH)) [13, 15]. In addition, the cooking of processed meat (fried, grilled, roasted, boiled and smoked), temperature and duration of cooking, type of fuel used for cooking, and proximity and direct contact with the heat source, can produce known or suspected carcinogens, including heterocyclic aromatic amines (HAA) and PAH [13, 15]. While the connection between consuming red meat and processed meat and developing cancer appears rather consistent, the connection between consuming poultry and fish and developing cancer is much less clear. Fish consumption has, however, been linked to anti-inflammatory and anticarcinogenic effects of long-chain n-3 fatty acids and could thus be beneficial for inhibiting carcinogenesis [16].

The IARC Monographs Working Group has evaluated the consumption of red meat and processed meat with respect to carcinogenicity to humans. Based on epidemiological evidence, it concluded that there are convincing associations between the consumption of red meat and cancer, particularly for cancers of the colorectum, pancreas and prostate [13]. In addition, the consumption of processed meat has been linked to cancers of the colorectum and stomach [13].

Outcome measure
CUP is defined as a metastasised epithelial malignancy with no identifiable primary tumour origin after cytological and/or histological verification during a patient’s lifetime. This CUP definition only includes epithelial malignancies (ICD-O-3 M-8000–M-8570), which excludes for example sarcoma, lymphoma, mesothelioma, and melanoma.

Follow-up
Cancer follow-up was established through annual record linkage with the Netherlands Cancer Registry (NCR) and the Dutch Pathology Registry (PALGA) [19]. Information regarding the site of metastasis was obtained from the NCR, but this data was only partially available and, therefore, supplementary information was retrieved from the pathology excerpts provided by PALGA. These pathology excerpts were also used to determine whether cytological and/or histological confirmed cases had been correctly categorised in the data received from the NCR. After 20.3 years of follow-up (17 Sep 1986 until 31 Dec 2006), data was available for a total of 1353 potential CUP cases, and a subcohort of 4774 participants after removing members who reported a history of cancer (except for skin cancer) at baseline.
excluding CUP cases without microscopical confirmation or non-epithelial histology, a total of 1073 CUP cases remained. CUP cases were further subdivided according to histology, according to the number of metastases (multiple metastases of the same type were counted as one metastatic site, for example, bone metastases in hip and vertebra were counted as one), according to localisation of metastasis (up to four locations), and according to survival duration. Participants were removed from the analysis if there was incomplete or inconsistent dietary data, or if there were selected confounders with missing values. As a result, 899 CUP cases and 4111 subcohort members were available for assessment (see Fig. 1).

**Questionnaire data**

Participants completed a self-administered questionnaire that included detailed questions on dietary habits, lifestyle, and other cancer risk factors. A 150 item semi quantitative food-frequency questionnaire was used that concentrated on the habitual consumption of food and beverages during the year preceding baseline [20, 21]. The food frequency questionnaire had been validated against a 9 day diet record and was tested for reproducibility in the NLCS [22, 23]. The Spearman correlation coefficients for the validity of red meat, processed meat, and fish, as investigated by the questionnaire were, 0.46, 0.54 and 0.53, respectively, compared to the results of the 9 day diet record [22]. The questionnaire contained 14 items on the consumption of meat as the main meal, five items on the consumption of meat used as a sandwich filling, and three items on the consumption of fish. Meats were grouped into red meat (overall), processed meat, and poultry. Red meat included beef, pork, minced meat (beef and pork), liver, and other meats (e.g., horsemeat, lamb). Processed meat (meat items that had undergone some form of preservation with nitrite salt, fermentation, or smoking) included ham, bacon, smoked beef, pork loin roll, and other sliced cold meats (e.g., sausages). Poultry included chicken and turkey. Fish consumption was measured in relation to the main meal, lunch, or as a snack between meals.

**Statistical analysis**

Person-years at risk were calculated from baseline (17 Sep 1986) until CUP diagnosis, death, emigration, loss to follow-up, or end of follow-up (31 Dec 2006), whichever occurred first. General characteristics were presented for subcohort members and CUP cases with frequencies (%) for categorical variables and means including standard deviations for continuous variables. Based on the distribution of the subcohort, participants were compared using quartiles (Q) or categories of red meat, processed meat, poultry, and fish consumption. For continuous analyses, increments of 50 g per day were used for red meat, beef, pork, minced meat, and poultry consumption, and increments of 25 g per day were used for liver, processed meat, and fish consumption.

The predefined confounders included age at baseline (years, continuous), sex (male/female), alcohol consumption (ethanol intake per day), cigarette smoking status (never/ever), cigarette smoking frequency (number of cigarettes smoked per day), cigarette smoking duration (number of years smoking), and total energy intake (kcal/day). The potential confounders included body mass index (BMI) at baseline (kg/m²), non-occupational physical activity (< 30 min/day, 30–60 min/day, 60–90 min/day and > 90 min/day), socio-economic status (highest level of education); diabetes (yes/no); history of cancer in a first degree relative (yes/no); and vegetable and fruit consumption (grams per day). Variables were considered a confounder if they changed the HR by > 10%. Accordingly, none of the potential confounders were included in the final model. No mutual adjustments were conducted between meat groups, as there

Fig. 1 Netherlands cohort study
was insufficient scientific evidence to conclude that they were related to CUP development.

Cox proportional hazards models were utilised to estimate age and sex adjusted, and multivariable-adjusted hazard ratios (HRs) with 95% confidence intervals (CIs). Standard errors were calculated using the robust Huber–White sandwich estimator to account for additional variance introduced by sampling from the full cohort [24]. The proportional hazards assumption was tested using the scaled Schoenfeld residuals [25]. In cases where the assumption had been violated, a time-varying covariate for that variable was added to the model where appropriate. Ordinal exposure variables were fitted as continuous variables in trend analyses. Wald tests and cross-product terms were used to evaluate possible multiplicative interaction between sex in relation to meat consumption and CUP risk, or between cigarette smoking status in relation to meat consumption and CUP risk. Analyses were conducted using Stata version 15. p values were considered statistically significant if $p < 0.05$.

Three sensitivity analyses were conducted, the first of which was restricted to histologically verified CUP cases only, since it is more likely that those cases had undergone extensive diagnostic investigation(s) to rule out the primary tumour origin. For those patients who received cytological verification alone, other factors may have played a role in the decision to refrain from further diagnostic investigation, such as age, comorbidities, performance status, localisation of metastasis, or the patient’s decision. The second sensitivity analysis was performed after the first 2 years of follow-up had been excluded so as to check for potential reverse causality bias as a result of preclinical cancer at baseline. Reverse causality bias may occur if participants change their dietary behaviour as a result of symptoms of preclinical cancer, whereas we are interested to see if dietary behaviour reduces or increases CUP risk. In the third sensitivity analysis, the first 10 years of follow-up (< 1996) were compared to the last 10 years of follow-up (≥ 1996), as to see whether associations between meat consumption and CUP risk differed over time.

**Results**

The statistical analyses of this study are based on 899 incident CUP cases and 4111 subcohort members with complete and consistent dietary data.

CUP cases appeared to consume slightly more red meat (overall), processed meat, and fish than subcohort members (90.8 g/day vs. 86.9 g/day and 15.0 g/day vs. 13.1 g/day and 14.1 g/day vs. 12.9 g/day, respectively) (Table 1). By contrast, subcohort members ate slightly more poultry than CUP cases (13.5 g/day vs. 12.9 g/day). The comparison between CUP cases and subcohort members appeared to be confounded by sex with respect to consuming red meat (overall) and processed meat. Male CUP cases consumed more red meat (overall) than male subcohort members (95.1 g/day vs. 93.4 g/day). Female CUP cases consumed more red meat (overall) than female subcohort members (83.5 g/day vs. 80.6 g/day). In addition, male CUP cases ate slightly more processed meats than male subcohort members (16.4 g/day vs. 15.8 g/day). Female CUP cases also ate more processed meats than female subcohort members (12.7 g/day vs. 10.4 g/day). Neither poultry consumption nor fish consumption appeared to be confounded by sex.

Findings of the age and sex adjusted analyses were comparable to those of the multivariable adjusted analyses, which were additionally adjusted for alcohol consumption, cigarette smoking variables (status, frequency, duration), and total energy intake. Hence, only the results of the multivariable analyses are described below. In general, we observed no association between red meat (overall) consumption and CUP risk (HR for Q4 vs. Q1 1.04, 95% CI 0.83–1.30, $P_{\text{trend}} = 0.31$) (Table 2). We observed an increased risk between beef consumption and CUP, for which a statistically significant trend was found (HR for Q4 vs. Q1 1.22, 95% CI 0.99–1.52, $P_{\text{trend}} = 0.02$). A statistically significant association was also observed between processed meat consumption and CUP risk (HR for Q4 vs. Q1 1.40, 95% CI 1.12–1.75, $P_{\text{trend}} = 0.006$). No association was found between poultry consumption and CUP risk (HR for C4 vs. C1 0.97, 95% CI 0.79–1.21, $P_{\text{trend}} = 0.28$). For fish consumption, we observed an increased CUP risk, but it was not statistically significant (HR for Q4 vs. Q1 1.25, 95% CI 0.99–1.57, $P_{\text{trend}} = 0.29$).

As described above, meat consumption differed markedly between men and women concerning both red meat (overall) and processed meat. Therefore, we stratified the analyses based on sex (Table 3). For beef consumption and CUP risk in men alone, the association attenuated and the trend was no longer statistically significant (HR for Q4 vs. Q1 1.12, 95% CI 0.85–1.47, $P_{\text{trend}} = 0.31$). Conversely, for beef consumption and CUP risk in women alone, the association became stronger and was statistically significant (HR for Q4 vs. Q1 1.47, 95% CI 1.04–2.07, $P_{\text{trend}} = 0.004$). For processed meat consumption and CUP risk in men alone, the association slightly attenuated and was no longer statistically significant (HR for Q4 vs. Q1 1.33, 95% CI 0.99–1.79, $P_{\text{trend}} = 0.15$). Yet, the association appeared to be more pronounced in women and remained statistically significant (HR for Q4 vs. Q1 1.53, 95% CI 1.08–2.16, $P_{\text{trend}} = 0.001$).

Furthermore, we checked whether there was a potential for residual confounding by cigarette smoking status and the association between meat consumption and CUP risk. We observed that the associations between beef and processed meat consumption and CUP risk increased when comparing current smokers to never smokers in women (data not shown). It should, however, be acknowledged that there were
Table 1 Characteristics of cancer of unknown primary cases and subcohort members in The Netherlands cohort study

| Characteristic | Subcohort members (n=4111) | Cancer of Unknown Primary cases (n=899) |
|---------------|-----------------------------|----------------------------------------|
|               | n  | (%) | n  | (%) |
| Age at baseline (years) |                |                |
| 55–59     | 1605 | 39.0 | 276 | 30.7 |
| 60–64     | 1402 | 34.1 | 349 | 38.8 |
| 65–69     | 1104 | 26.9 | 274 | 30.5 |
| Sex        |                |                |
| Men        | 2022 | 49.2 | 568 | 63.2 |
| Women     | 2089 | 50.8 | 331 | 36.8 |
| Red meat consumption (g/day) |                |                |
| Men        | 86.9 (40.5) | 90.8 (40.3) | |
| Women     | 93.4 (41.6) | 95.1 (41.6) | |
| Processed meat consumption (g/day) |                |                |
| Men        | 13.1 (14.6) | 15.0 (15.5) | |
| Women     | 15.8 (16.7) | 16.4 (15.6) | |
| Poultry consumption (g/day) |                |                |
| Men        | 13.5 (15.1) | 12.9 (14.4) | |
| Women     | 13.6 (14.7) | 13.3 (15.3) | |
| Fish consumption (g/day) |                |                |
| Men        | 12.9 (15.3) | 14.1 (17.9) | |
| Women     | 14.1 (16.6) | 15.5 (20.2) | |
| Ethanol intake (g/day)a |                |                |
| Abstainers | 975 | 23.7 | 170 | 18.9 |
| < 5        | 1179 | 28.7 | 233 | 25.9 |
| 5– < 15    | 938 | 22.8 | 204 | 22.7 |
| 15– < 30   | 651 | 15.8 | 143 | 15.9 |
| ≥ 30       | 368 | 9.0 | 149 | 16.6 |
| Cigarette smoking status |                |                |
| Never smokers | 1517 | 36.9 | 249 | 27.7 |
| Ex-smokers | 1479 | 36.0 | 317 | 35.3 |
| Current smokers | 1115 | 27.1 | 333 | 37.0 |
| Frequency of cigarette smoking (N/day), mean (SD)b | 15.7 (10.1) | 17.9 (10.4) |
| Duration of cigarette smoking (years), mean (SD)b | 31.8 (12.1) | 35.4 (11.7) |
| Body mass index at baseline (kg/m²), mean (SD) | 25.0 (3.1) | 24.9 (3.0) |
| Non-occupational physical activity (min/day) |                |                |
| ≤ 30       | 844 | 20.8 | 182 | 20.5 |
| > 30–60    | 1269 | 31.2 | 274 | 30.9 |
| > 60–90    | 857 | 21.1 | 160 | 18.0 |
| > 90       | 1093 | 26.9 | 271 | 30.6 |
| Level of education (years of education) |                |                |
| Primary    | 1154 | 28.2 | 237 | 26.6 |
| Lower vocational | 899 | 22.0 | 192 | 21.6 |
| Secondary and medium vocational | 1457 | 35.6 | 329 | 36.9 |
| University and higher vocational | 582 | 14.2 | 133 | 14.9 |
| Diabetes   |                |                |
| Yes        | 140 | 3.4 | 36 | 4.0 |
| First grade family history of cancer |                |                |
| Yes        | 1870 | 45.5 | 436 | 48.5 |

a In consumers only
b In users only
Table 2: Hazard ratios and 95% confidence intervals for meat consumption and cancer of unknown primary risk in the Netherlands cohort study

| Subcohort members (n=4111) | Cancer of Unknown Primary cases (n=899) |
|-----------------------------|---------------------------------------|
| Categorical median (grams per day) | Person time at risk (years) | Cases | Age and sex adjusteda | Multivariable adjustedb |
| Men | Women |  | | HR | 95% CI | HR | 95% CI |

**Red meat (overall)**

| | Men | Women | | | | |
|---|---|---|---|---|---|---|
| Q1 | 50 | 41 | 17 433 | 205 | 1 | Reference | 1 | Reference |
| Q2 | 79 | 68 | 17 338 | 224 | 1 | 1.14 | (0.92–1.41) | 1 | 1.11 | (0.89–1.37) |
| Q3 | 100 | 88 | 17 184 | 248 | 1 | 1.26 | (1.02–1.55) | 1 | 1.21 | (0.98–1.49) |
| Q4 | 139 | 125 | 17 548 | 222 | 1 | 1.13 | (0.91–1.40) | 1 | 1.04 | (0.83–1.30) |
| p for trendc | | | | | | | | 0.08 | 0.31 |
| Continuous, 50 g per day increments | | | | | | | | 69 503 | 899 | 1.08 | (0.99–1.18) | 1.05 | (0.96–1.15) |

**Beef**

| | Men | Women | | | | |
|---|---|---|---|---|---|---|
| Q1 | 4 | 3 | 17 293 | 199 | 1 | Reference | 1 | Reference |
| Q2 | 16 | 14 | 17 065 | 208 | 1 | 1.05 | (0.84–1.30) | 1 | 1.03 | (0.82–1.28) |
| Q3 | 30 | 26 | 17 986 | 231 | 1 | 1 | (0.89–1.36) | 1 | 1.08 | (0.87–1.34) |
| Q4 | 53 | 47 | 17 160 | 261 | 1 | 1.25 | (1.01–1.54) | 1 | 1.22 | (0.99–1.52) |
| p for trendc | | | | | | | | 0.01 | 0.02 |
| Continuous, 50 g per day increments | | | | | | | | 69 503 | 899 | 1.21 | (1.04–1.41) | 1.21 | (1.03–1.42) |

**Pork**

| | Men | Women | | | | |
|---|---|---|---|---|---|---|
| Q1 | 9 | 5 | 17 461 | 214 | 1 | Reference | 1 | Reference |
| Q2 | 28 | 23 | 17 308 | 250 | 1 | 1.21 | (0.98–1.48) | 1 | 1.14 | (0.93–1.41) |
| Q3 | 44 | 40 | 17 288 | 216 | 1 | 1.07 | (0.86–1.32) | 1 | 1.01 | (0.81–1.25) |
| Q4 | 74 | 65 | 17 445 | 219 | 1 | 1.09 | (0.88–1.35) | 0.99 | (0.79–1.23) |
| p for trendc | | | | | | | | 0.65 | 0.78 |
| Continuous, 50 g per day increments | | | | | | | | 69 503 | 899 | 1.03 | (0.91–1.16) | 0.98 | (0.86–1.12) |

**Minced meat**

| | Men | Women | | | | |
|---|---|---|---|---|---|---|
| Q1 | 3 | 0 | 16 932 | 237 | 1 | Reference | 1 | Reference |
| Q2 | 12 | 10 | 17 106 | 239 | 1 | 1.01 | (0.82–1.24) | 1 | 1.01 | (0.82–1.24) |
| Q3 | 21 | 18 | 17 801 | 207 | 0.87 | (0.70–1.07) | 0.86 | (0.70–1.07) |
| Q4 | 38 | 33 | 17 664 | 216 | 0.90 | (0.73–1.11) | 0.91 | (0.74–1.13) |
| p for trendc | | | | | | | | 0.23 | 0.22 |
| Continuous, 50 g per day increments | | | | | | | | 69 503 | 899 | 0.87 | (0.69–1.09) | 0.86 | (0.68–1.09) |

**Liver**

| | Men | Women | | | | |
|---|---|---|---|---|---|---|
| C1 | 0 | 0 | 44 786 | 585 | 1 | Reference | 1 | Reference |
| C2 | 4 | 3 | 24 716 | 314 | 0.99 | (0.84–1.16) | 0.96 | (0.82–1.12) |
| p for trendc | | | | | | | | 0.92 | 0.87 |
| Continuous, 25 g per day increments | | | | | | | | 69 503 | 899 | 1.02 | (0.65–1.63) | 0.96 | (0.59–1.56) |

**Poultry**

| | Men | Women | | | | |
|---|---|---|---|---|---|---|
| C1 | 0 | 0 | 16 123 | 203 | 1 | Reference | 1 | Reference |
| C2 | 5 | 5 | 17 045 | 230 | 1 | 1.12 | (0.90–1.38) | 1 | 1.13 | (0.91–1.41) |
| C3 | 13 | 13 | 16 570 | 227 | 1 | 1.12 | (0.90–1.38) | 1 | 1.10 | (0.88–1.37) |
| C4 | 23 | 23 | 19 766 | 239 | 0.98 | (0.79–1.21) | 0.97 | (0.79–1.21) |
| p for trendc | | | | | | | | 0.31 | 0.28 |
| Continuous, 50 g per day increments | | | | | | | | 69 503 | 899 | 0.88 | (0.68–1.13) | 0.86 | (0.66–1.13) |

**Processed meat**

| | Men | Women | | | | |
|---|---|---|---|---|---|---|
| Q1 | 1 | 0 | 17 060 | 201 | 1 | Reference | 1 | Reference |
| Q2 | 8 | 4 | 17 949 | 216 | 1 | 1.09 | (0.88–1.35) | 1 | 1.07 | (0.86–1.33) |
| Q3 | 16 | 11 | 17 095 | 222 | 1 | 1.16 | (0.93–1.44) | 1 | 1.14 | (0.91–1.42) |
| Q4 | 33 | 22 | 17 398 | 260 | 1 | 1.38 | (1.12–1.70) | 1 | 1.40 | (1.12–1.75) |
| p for trendc | | | | | | | | 0.006 | 0.006 |
fewer cases available in the categories due to the stratification for both sex and cigarette smoking status. Our observations suggest that residual confounding by cigarette smoking status is unlikely in women.

We observed no multiplicative interactions between sex and the consumption of red meat (overall), beef, pork, minced meat, liver, processed meat, poultry, or fish in relation to CUP risk ($P_{\text{interaction}} = 0.64, 0.55, 0.22, 0.19, 0.41, 0.52, 0.11,$ and $0.22,$ respectively). In addition, no multiplicative interactions were observed between cigarette smoking status and the consumption of red meat (overall), beef, pork, minced meat, liver, processed meat, poultry, or fish in relation to CUP risk ($P_{\text{interaction}} = 0.27, 0.88, 0.22, 0.56, 0.14, 0.24, 0.88,$ and $0.80,$ respectively).

Results from the first sensitivity analysis with restriction to histologically verified CUP cases alone, for whom extended diagnostic methods are more expected (compared to cytologically verified CUP cases), indicate that the findings are similar to those of the overall multivariable analyses except for beef consumption and CUP risk (HR for Q4 vs. Q1 1.16, 95% CI 0.91–1.49, $P_{\text{trend}} = 0.21$), possibly due to fewer cases. We presume that the results of the overall multivariable analyses represent CUP cases with or without an extensive diagnostic work-up. Our secondary sensitivity analysis, in which the first 2 years of follow-up were excluded so as to check for potential reverse causality bias, also demonstrate similar findings to those observed in the complete analysis (data not shown). In our third sensitivity analysis, after splitting the follow-up time to compare the first 10 years of follow-up to the last 10 years of follow-up, we observed that the association between beef consumption and CUP risk was the highest in the first 10 years of follow-up, whereas it attenuated in the last 10 years of follow-up. On the other hand, for processed meat consumption and CUP risk, no association was found in the first 10 years of follow-up, while there was a positive statistically significant association in the last 10 years of follow-up.

**Discussion**

In this detailed investigation of meat consumption and CUP risk, we found that beef and processed meat consumption were positively associated with the development of CUP in women. We found a non-significant positive association between processed meat consumption and CUP risk, no association was found in the first 10 years of follow-up, while there was a positive statistically significant association in the last 10 years of follow-up.
Table 3: Hazard ratios and 95% confidence intervals for meat consumption and Cancer of Unknown Primary risk in the Netherlands Cohort Study, stratified for sex

|                | Men only |                                                                 | Women only |                                                                 |
|----------------|----------|------------------------------------------------------------------|------------|------------------------------------------------------------------|
|                | Categorical median (grams per day) | Subcohort members (n=2022) | Cancer of Unknown Primary cases (n=568) | Person time at risk (years) | Cases | Multivariable adjusted<sup>a</sup> | Person time at risk (years) | Cases | Multivariable adjusted<sup>a</sup> |
| Red meat (overall) |          |                                                                 |            |                                                                  |       |                                  |            |       |                                  |
| Q1             | 50       | 8 143                                                            | 135        | 1 Reference                                                      | 41     | 9 290                             | 70         | 1 Reference                           |
| Q2             | 79       | 7 692                                                            | 142        | 1.07 (0.81–1.42)                                                | 68     | 9 376                             | 82         | 1.19 (0.85–1.68)                        |
| Q3             | 100      | 7 856                                                            | 146        | 1.10 (0.83–1.44)                                                | 88     | 9 328                             | 102        | 1.43 (1.03–2.00)                       |
| Q4             | 139      | 8 222                                                            | 145        | 1.01 (0.76–1.35)                                                | 125    | 9 326                             | 77         | 1.09 (0.77–1.54)                       |
| p for trend<sup>b</sup> |          |                                                                  |            |                                                                  |       |                                  |            |       |                                  |
| Continuous, 50 g per day increments | 32 182   | 568                                                              | 1.02 (0.91–1.16) | 37 320 | 331                             | 1.10 (0.95–1.27) | |
| Beef |          |                                                                 |            |                                                                  |       |                                  |            |       |                                  |
| Q1             | 4        | 7 988                                                            | 133        | 1 Reference                                                      | 3      | 9 305                             | 66         | 1 Reference                           |
| Q2             | 16       | 8 003                                                            | 134        | 0.97 (0.73–1.29)                                                | 14     | 9 062                             | 74         | 1.15 (0.81–1.65)                       |
| Q3             | 30       | 8 116                                                            | 138        | 0.97 (0.73–1.29)                                                | 26     | 9 870                             | 93         | 1.34 (0.95–1.88)                       |
| Q4             | 53       | 8 076                                                            | 163        | 1.12 (0.85–1.47)                                                | 47     | 9 083                             | 98         | 1.47 (1.04–2.07)                       |
| p for trend<sup>b</sup> |          |                                                                  |            |                                                                  |       |                                  |            |       |                                  |
| Continuous, 50 g per day increments | 32 182   | 568                                                              | 1.11 (0.91–1.37) | 37 320 | 331                             | 1.41 (1.11–1.78) | |
| Pork |          |                                                                 |            |                                                                  |       |                                  |            |       |                                  |
| Q1             | 9        | 8 157                                                            | 128        | 1 Reference                                                      | 5      | 9 305                             | 86         | 1 Reference                           |
| Q2             | 28       | 8 037                                                            | 151        | 1.13 (0.85–1.49)                                                | 23     | 9 271                             | 99         | 1.16 (0.85–1.59)                       |
| Q3             | 44       | 7 787                                                            | 137        | 1.07 (0.80–1.34)                                                | 40     | 9 502                             | 79         | 0.90 (0.64–1.25)                       |
| Q4             | 74       | 8 202                                                            | 152        | 1.15 (0.86–1.53)                                                | 65     | 9 244                             | 67         | 0.75 (0.53–1.06)                       |
| p for trend<sup>b</sup> |          |                                                                  |            |                                                                  |       |                                  |            |       |                                  |
| Continuous, 50 g per day increments | 32 182   | 568                                                              | 1.06 (0.90–1.25) | 37 320 | 331                             | 0.83 (0.66–1.04) | |
| Minced meat |          |                                                                 |            |                                                                  |       |                                  |            |       |                                  |
| Q1             | 3        | 7 829                                                            | 162        | 1 Reference                                                      | 0      | 9 103                             | 75         | 1 Reference                           |
| Q2             | 12       | 8 049                                                            | 151        | 0.92 (0.70–1.20)                                                | 10     | 9 056                             | 88         | 1.20 (0.86–1.68)                       |
| Q3             | 21       | 8 138                                                            | 127        | 0.76 (0.58–1.00)                                                | 18     | 9 663                             | 80         | 1.07 (0.76–1.52)                       |
| Q4             | 38       | 8 166                                                            | 128        | 0.78 (0.59–1.03)                                                | 33     | 9 498                             | 88         | 1.19 (0.85–1.67)                       |
| p for trend<sup>b</sup> |          |                                                                  |            |                                                                  |       |                                  |            |       |                                  |
| Continuous, 50 g per day increments | 32 182   | 568                                                              | 0.76 (0.56–1.03) | 37 320 | 331                             | 1.13 (0.77–1.64) | |
Table 3 (continued)

| Liver | Men only | Cancer of Unknown Primary cases | Women only | Cancer of Unknown Primary cases |
|-------|----------|---------------------------------|------------|---------------------------------|
|       | Categorical median (grams per day) | Subcohort members (n=2022) | Cases | Multivariable adjusted<sup>a</sup> | Person time at risk (years) | n | HR | 95% CI | Person time at risk (years) | n | HR | 95% CI |
|       |       | Cancer of Unknown Primary cases (n=568) | | | | n | HR | 95% CI | | n | HR | 95% CI |
|       |       | | | | | | | | | | | |
| Liver | C1 | 0 | 19 695 | 362 | 1 | Reference | 0 | 25 092 | 223 | 1 | Reference | 0.88 |
|       | C2 | 4 | 12 488 | 206 | 0.92 | (0.75–1.12) | 3 | 12 229 | 108 | 1.03 | (0.80–1.33) | 0.98 |
|       | p for trend | | | | | | | | | | | 0.88 |
|       | Continuous, 25 g per day increments | 32 182 | 568 | 0.95 | (0.52–1.75) | 37 320 | 331 | 0.99 | (0.45–2.19) |
| Poultry | C1 | 0 | 7 542 | 121 | 1 | Reference | 0 | 8 580 | 82 | 1 | Reference | 0.87 |
|       | C2 | 5 | 7 248 | 151 | 1.33 | (1.00–1.76) | 5 | 9 797 | 79 | 0.87 | (0.62–1.21) | 0.11 |
|       | C3 | 13 | 8 044 | 139 | 1.08 | (0.81–1.44) | 13 | 8 526 | 88 | 1.14 | (0.82–1.59) | 0.46 |
|       | C4 | 23 | 9 348 | 157 | 1.07 | (0.80–1.41) | 23 | 10 417 | 82 | 0.84 | (0.60–1.17) | 1.00 |
|       | p for trend | | | | | | | | | | | 0.11 |
|       | Continuous, 50 g per day increments | 32 182 | 568 | 0.97 | (0.67–1.40) | 37 320 | 331 | 0.73 | (0.50–1.07) |
| Processed meat | Q1 | 1 | 8 075 | 135 | 1 | Reference | 0 | 8 986 | 66 | 1 | Reference | 0.15 |
|       | Q2 | 8 | 7 974 | 126 | 0.98 | (0.74–1.30) | 4 | 9 975 | 90 | 1.25 | (0.88–1.76) | 0.001 |
|       | Q3 | 16 | 8 097 | 149 | 1.15 | (0.87–1.53) | 11 | 8 998 | 73 | 1.12 | (0.78–1.61) | 0.99 |
|       | Q4 | 33 | 8 036 | 158 | 1.33 | (0.99–1.79) | 22 | 9 362 | 102 | 1.53 | (1.08–2.16) | 0.22 |
|       | p for trend<sup>2</sup> | | | | | | | | | | | 0.001 |
|       | Continuous, 25 g per day increments | 32 182 | 568 | 1.11 | (0.96–1.28) | 37 320 | 331 | 1.50 | (1.17–1.93) |
| Fish | Q1 | 0 | 8 231 | 119 | 1 | Reference | 0 | 11 617 | 89 | 1 | Reference | 0.22 |
|       | Q2 | 5 | 7 256 | 144 | 1.36 | (1.02–1.81) | 5 | 8 336 | 72 | 1.18 | (0.84–1.66) | 0.99 |
|       | Q3 | 15 | 10 054 | 169 | 1.12 | (0.85–1.48) | 15 | 10 991 | 117 | 1.43 | (1.05–1.95) | 0.99 |
|       | Q4 | 32 | 6 642 | 136 | 1.31 | (0.98–1.76) | 28 | 6 377 | 53 | 1.10 | (0.76–1.61) | 0.99 |
|       | p for trend<sup>2</sup> | | | | | | | | | | | 0.99 |
|       | Continuous, 25 g per day increments | 32 182 | 568 | 1.10 | (0.95–1.27) | 37 320 | 331 | 1.00 | (0.82–1.21) |

<sup>a</sup>Analyses were adjusted for age at baseline (years), alcohol consumption, cigarette smoking status (never/ever), cigarette smoke frequency (continuous, centered), cigarette smoking duration (continuous, centered), and total energy intake (kcal/day)

<sup>b</sup>Tests for dose–response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazards model
### Table 4: Hazard ratios and 95% confidence intervals for meat consumption and cancer of unknown primary risk in the Netherlands cohort study, stratified for cigarette smoking status

| Smoking status = never | Smoking status = ex | Smoking status = current |
|------------------------|---------------------|-------------------------|
| Subcohort Cancer of Unknown Primary cases (n=249) | Subcohort Cancer of Unknown Primary cases (n=317) | Subcohort Cancer of Unknown Primary cases (n=333) |
| Person time at risk (years) n | Person time at risk (years) n | Person time at risk (years) n |
| **Cases** | **Age and sex adjusted** | **Multivariable adjusted** | **Cases** | **Age and sex adjusted** | **Multivariable adjusted** | **Cases** | **Age and sex adjusted** | **Multivariable adjusted** |
| **HR** | **95% CI** | **HR** | **95% CI** | **HR** | **95% CI** | **HR** | **95% CI** | **HR** | **95% CI** |
| **Q1** | 7 381 | 59 | 1 | Reference | 1 | Reference | 6 067 | 73 | 1 | Reference | 1 | Reference | 3 895 | 73 | 1 | Reference | 1 | Reference |
| **Q2** | 6 603 | 71 | 1.39 | (0.95–2.02) | 1.39 | (0.95–2.04) | 6 476 | 84 | 1.15 | (0.81–1.63) | 1.15 | (0.81–1.65) | 4 259 | 69 | 0.88 | (0.60–1.29) | 0.84 | (0.57–1.24) |
| **Q3** | 6 891 | 74 | 1.40 | (0.96–2.04) | 1.43 | (0.98–2.08) | 6 071 | 77 | 1.08 | (0.76–1.55) | 1.00 | (0.70–1.45) | 4 221 | 97 | 1.27 | (0.88–1.82) | 1.22 | (0.84–1.75) |
| **Q4** | 6 403 | 45 | 0.93 | (0.61–1.42) | 0.96 | (0.62–1.48) | 6 134 | 83 | 1.18 | (0.83–1.69) | 1.01 | (0.69–1.47) | 5 011 | 94 | 1.09 | (0.76–1.57) | 1.05 | (0.72–1.53) |
| **p for trend** | 0.74 | 0.59 | 1.05 | (0.89–1.23) | 24 749 | 317 | 1.03 | (0.89–1.19) | 0.95 | (0.81–1.12) | 17 477 | 333 | 1.14 | (0.97–1.34) | 1.15 | (0.97–1.36) |
| **Continuous, 50 g per day increments** | 27 278 | 249 | 1.03 | (0.88–1.20) | 24 749 | 317 | 1.03 | (0.89–1.19) | 0.95 | (0.81–1.12) | 17 477 | 333 | 1.14 | (0.97–1.34) | 1.15 | (0.97–1.36) |
| **Beef** | 6 475 | 48 | 1 | Reference | 1 | Reference | 6 205 | 70 | 1 | Reference | 1 | Reference | 4 612 | 81 | 1 | Reference | 1 | Reference |
| **Q1** | 6 475 | 48 | 1 | Reference | 1 | Reference | 6 205 | 70 | 1 | Reference | 1 | Reference | 4 612 | 81 | 1 | Reference | 1 | Reference |
| **Q2** | 6 859 | 64 | 1.23 | (0.82–1.85) | 1.24 | (0.83–1.85) | 5 929 | 67 | 1.03 | (0.71–1.49) | 1.01 | (0.69–1.48) | 4 277 | 77 | 0.94 | (0.65–1.36) | 0.90 | (0.62–1.31) |
| **Q3** | 7 221 | 70 | 1.30 | (0.87–1.94) | 1.31 | (0.88–1.95) | 6 227 | 79 | 1.09 | (0.76–1.56) | 1.08 | (0.75–1.56) | 4 487 | 82 | 0.98 | (0.68–1.40) | 0.94 | (0.65–1.36) |
| **Q4** | 6 722 | 67 | 1.23 | (0.81–1.84) | 1.25 | (0.83–1.88) | 6 337 | 101 | 1.36 | (0.97–1.93) | 1.29 | (0.90–1.83) | 4 100 | 93 | 1.18 | (0.83–1.69) | 1.15 | (0.80–1.67) |
| **p for trend** | 0.25 | 0.21 | 0.05 | 0.12 | 0.18 | 0.12 | 17 477 | 333 | 1.21 | (0.91–1.61) | 1.22 | (0.91–1.63) | 0.998 |
| **Continuous, 50 g per day increments** | 27 278 | 249 | 1.19 | (0.89–1.59) | 24 749 | 317 | 1.27 | (1.00–1.60) | 1.21 | (0.95–1.54) | 17 477 | 333 | 1.21 | (0.91–1.61) | 1.22 | (0.91–1.63) |
| **Pork** | 7 206 | 67 | 1 | Reference | 1 | Reference | 6 488 | 79 | 1 | Reference | 1 | Reference | 3 768 | 68 | 1 | Reference | 1 | Reference |
| **Q1** | 7 206 | 67 | 1 | Reference | 1 | Reference | 6 488 | 79 | 1 | Reference | 1 | Reference | 3 768 | 68 | 1 | Reference | 1 | Reference |
| **Q2** | 6 850 | 71 | 1.14 | (0.79–1.64) | 1.14 | (0.79–1.65) | 6 253 | 78 | 1.06 | (0.74–1.50) | 0.98 | (0.69–1.40) | 4 205 | 101 | 1.35 | (0.93–1.94) | 1.27 | (0.88–1.84) |
| **Q3** | 6 731 | 67 | 1.13 | (0.78–1.64) | 1.14 | (0.78–1.65) | 5 944 | 78 | 1.16 | (0.81–1.66) | 1.11 | (0.77–1.59) | 4 614 | 71 | 0.85 | (0.57–1.24) | 0.82 | (0.56–1.22) |
| **Q4** | 6 491 | 44 | 0.77 | (0.51–1.17) | 0.78 | (0.52–1.19) | 6 064 | 82 | 1.15 | (0.81–1.64) | 0.97 | (0.67–1.41) | 4 890 | 93 | 1.17 | (0.81–1.70) | 1.10 | (0.75–1.62) |
| **p for trend** | 0.03 | 0.04 | 0.73 | 0.63 | 0.19 | 0.24 | 0.220 |
Table 4 (continued)

| Smoking status = never | Smoking status = ex | Smoking status = current |
|------------------------|---------------------|-------------------------|
| Subcohort members | Cancer of Unknown Primary cases (n=249) | Subcohort Cancer of Unknown Primary cases (n=317) | Subcohort Cancer of Unknown Primary cases (n=333) |
| Person time at risk (years) | Cases | Age and sex adjusted | Multivariable adjusted | Person time at risk (years) | Cases | Age and sex adjusted | Multivariable adjusted | Person time at risk (years) | Cases | Age and sex adjusted | Multivariable adjusted | p for interaction |
| Continuous, 50 grams per day increments | Minced meat | | | | | | | | | | | | |
| 27 278 | 249 | 0.77 (0.61–0.97) | 0.78 (0.61–0.99) | 24 749 | 317 | 1.04 (0.85–1.27) | 0.95 (0.76–1.18) | 17 477 | 333 | 1.15 (0.93–1.43) | 1.15 (0.91–1.44) | 0.056 |
| Q1 | 6 896 | 60 | 1 | Reference | 1.08 (0.73–1.60) | 6 440 | 96 | 1.06 (0.76–1.47) | 1.01 (0.72–1.42) | 4 334 | 91 | 1 | Reference | 0.560 |
| Q2 | 6 329 | 60 | 1.08 (0.73–1.60) | 6 440 | 96 | 1.06 (0.76–1.47) | 1.01 (0.72–1.42) | 4 334 | 91 | 1 | Reference | 0.560 |
| Q3 | 7 369 | 65 | 1.09 (0.74–1.59) | 6 183 | 63 | 0.72 (0.50–1.05) | 0.70 (0.48–1.02) | 4 248 | 79 | 0.87 (0.60–1.24) | 0.86 (0.60–1.25) | 0.03 |
| Q4 | 6 683 | 64 | 1.17 (0.79–1.71) | 6 423 | 72 | 0.79 (0.55–1.12) | 0.77 (0.54–1.10) | 4 559 | 80 | 0.82 (0.58–1.17) | 0.86 (0.60–1.24) | 0.03 |
| p for trend | 0.32 | 0.29 | 0.03 | 0.02 | 0.46 | 0.61 | 0.07 |
| Liver | | | | | | | | | | | | | |
| 27 278 | 249 | 1.25 (0.80–1.95) | 1.28 (0.82–2.01) | 24 749 | 317 | 0.64 (0.43–0.95) | 0.62 (0.41–0.93) | 17 477 | 333 | 0.87 (0.59–1.27) | 0.90 (0.62–1.33) | 0.057 |
| C1 | 18 432 | 167 | 1 | Reference | 1.06 (0.79–1.43) | 8 866 | 93 | 0.79 (0.60–1.04) | 0.76 (0.58–1.01) | 7 005 | 139 | 1.11 (0.85–1.44) | 1.09 (0.83–1.42) | 0.14 |
| C2 | 8 845 | 82 | 1.05 (0.78–1.40) | 8 866 | 93 | 0.79 (0.60–1.04) | 0.76 (0.58–1.01) | 7 005 | 139 | 1.11 (0.85–1.44) | 1.09 (0.83–1.42) | 0.14 |
| p for trend | 0.34 | 0.29 | 0.06 | 0.06 | 0.81 | 0.81 | 0.07 |
| Poultry | | | | | | | | | | | | | |
| 27 278 | 249 | 1.51 (0.65–3.52) | 1.60 (0.68–3.77) | 24 749 | 317 | 0.45 (0.19–1.03) | 0.42 (0.17–1.02) | 17 477 | 333 | 1.09 (0.56–2.10) | 1.09 (0.55–2.15) | 0.107 |
| C1 | 6 611 | 58 | 1 | Reference | 1 | Reference | 1 | Reference | 1 | Reference | 1 | Reference | 0.88 |
| C2 | 6 856 | 65 | 1.12 (0.76–1.68) | 6 754 | 72 | 1.06 (0.73–1.53) | 1.07 (0.74–1.56) | 4 434 | 93 | 1.13 (0.79–1.61) | 1.21 (0.84–1.75) | 0.07 |
| C3 | 6 156 | 62 | 1.19 (0.81–1.77) | 6 113 | 80 | 1.13 (0.78–1.62) | 1.13 (0.78–1.63) | 4 301 | 81 | 1.04 (0.72–1.50) | 1.02 (0.69–1.49) | 0.09 |
| C4 | 7 654 | 64 | 0.99 (0.67–1.48) | 7 376 | 95 | 1.11 (0.78–1.57) | 1.09 (0.76–1.56) | 4 735 | 80 | 0.86 (0.60–1.24) | 0.88 (0.60–1.29) | 0.09 |
| p for trend | 0.81 | 0.85 | 0.90 | 0.85 | 0.10 | 0.10 | 0.32 |
### Table 4 (continued)

| Smoking status = never | Smoking status = ex | Smoking status = current |
|------------------------|---------------------|-------------------------|
| **Subcohort members** | **Cancer of Unknown Primary cases** | **Cancer of Unknown Primary cases** |
| (n=1517)               | (n=249)             | (n=317)                 |
| Person time at risk    | Cases               | Person time at risk     | Cases               | Person time at risk | Cases               | Person time at risk | Cases               |
| (years)                |                      | (years)                 |                      | (years)             |                      | (years)             |                      |
| Continuous, 50 g per day increments |                      | Continuous, 50 g per day increments |                      | Continuous, 50 g per day increments |                      |
| Q1 7 109 53 1 Reference 1 Reference | 27 278 249 0.94 (0.58–1.52) 0.96 (0.59–1.54) | 24 749 317 1.03 (0.68–1.54) 1.04 (0.67–1.61) | 17 477 333 0.69 (0.44–1.07) 0.68 (0.43–1.07) |
| Q2 6 710 70 1.49 (1.01–2.21) 1.52 (1.02–2.26) | 6 661 66 0.76 (0.54–1.09) 0.77 (0.54–1.10) | 4 578 80 1.15 (0.78–1.70) 1.12 (0.76–1.67) |
| Q3 6 620 59 1.29 (0.86–1.94) 1.36 (0.89–2.07) | 5 965 83 1.04 (0.74–1.47) 1.02 (0.72–1.44) | 4 510 80 1.13 (0.77–1.67) 1.15 (0.77–1.71) |
| Q4 6 838 67 1.42 (0.95–2.11) 1.55 (1.00–2.39) | 5 791 79 1.09 (0.77–1.54) 1.06 (0.73–1.54) | 4 769 114 1.53 (1.06–2.22) 1.66 (1.12–2.46) |
| p for trend | 0.10 0.05 | 0.19 0.29 | 0.35 0.09 |

| Processed meat |                      |                      |
|----------------|---------------------|---------------------|
| Q1 7 109 53 1 Reference 1 Reference | 27 278 249 0.94 (0.58–1.52) 0.96 (0.59–1.54) | 24 749 317 1.03 (0.68–1.54) 1.04 (0.67–1.61) | 17 477 333 0.69 (0.44–1.07) 0.68 (0.43–1.07) |
| Q2 6 710 70 1.49 (1.01–2.21) 1.52 (1.02–2.26) | 6 661 66 0.76 (0.54–1.09) 0.77 (0.54–1.10) | 4 578 80 1.15 (0.78–1.70) 1.12 (0.76–1.67) |
| Q3 6 620 59 1.29 (0.86–1.94) 1.36 (0.89–2.07) | 5 965 83 1.04 (0.74–1.47) 1.02 (0.72–1.44) | 4 510 80 1.13 (0.77–1.67) 1.15 (0.77–1.71) |
| Q4 6 838 67 1.42 (0.95–2.11) 1.55 (1.00–2.39) | 5 791 79 1.09 (0.77–1.54) 1.06 (0.73–1.54) | 4 769 114 1.53 (1.06–2.22) 1.66 (1.12–2.46) |
| p for trend | 0.10 0.05 | 0.19 0.29 | 0.35 0.09 |

| Fish |                      |                      |
|-----|---------------------|---------------------|
| Q1 9 221 63 1 Reference 1 Reference | 27 278 249 0.94 (0.58–1.52) 0.96 (0.59–1.54) | 24 749 317 1.03 (0.68–1.54) 1.04 (0.67–1.61) | 17 477 333 0.69 (0.44–1.07) 0.68 (0.43–1.07) |
| Q2 5 865 59 1.56 (1.06–2.30) 1.58 (1.07–2.32) | 5 983 79 1.13 (0.79–1.63) 1.12 (0.77–1.62) | 3 744 78 1.22 (0.84–1.76) 1.26 (0.86–1.83) |
| Q3 7 860 80 1.52 (1.06–2.18) 1.54 (1.07–2.22) | 8 109 109 1.22 (0.87–1.71) 1.14 (0.80–1.62) | 5 076 97 1.10 (0.78–1.56) 1.06 (0.74–1.53) |
| Q4 4 331 47 1.57 (1.04–2.38) 1.63 (1.07–2.49) | 4 570 62 1.20 (0.82–1.77) 1.16 (0.78–1.72) | 4 117 80 1.13 (0.79–1.64) 1.06 (0.73–1.55) |
| p for trend | 0.10 0.05 | 0.19 0.29 | 0.35 0.09 |

| p for interaction | 0.239 | 0.722 | 0.804 |

---

*a* Analyses were adjusted for age at baseline (years) and sex  
*b* Analyses were adjusted for age at baseline (years), sex, alcohol consumption, and total energy intake (kcal/day)  
*c* Interactions were calculated with respect to smoking status in relation to the meat variable of interest and CUP risk  
*d* Tests for dose–response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazards model
by contrast, we have investigated the association between meat consumption and CUP risk in greater detail by assessing combined groups of meats, such as red meat, processed meat, poultry, and fish, as well as individual meat items. We have found that beef and processed meat consumption are significantly associated with an increased CUP risk, but that red meat (overall), poultry, and fish consumption do not appear to be associated with CUP risk. Consequently, while our study confirms the findings of the Australian cohort study in indicating no association between red meat (overall) consumption and CUP risk, we do observe an association between beef and processed meat consumption and CUP risk [11]. The consumption of red and processed meat has been linked to colorectal cancer in previous epidemiological studies (probable increasing risk and convincing increasing risk, respectively) [26]. It also known that colorectal cancer predominantly metastasises to the liver via the portal circulation [27]; therefore, we have conducted an additional analysis to study whether the association between meat consumption is stronger in CUP patients with metastases located in the liver. We found the association between processed meat consumption and CUP risk in patients with a liver metastasis to be increased (per 25 g per day increment HR 1.34, 95% CI 1.14–1.58, \( P_{\text{trend}} = 0.001 \)) compared to the result of the overall analysis (per 25 g per day increment HR 1.19, 95% CI 1.05–1.34, \( P_{\text{trend}} = 0.006 \)). In addition, based on data obtained from the NCR, 36.1% of the primary tumours that metastasised to the liver, originated in the colorectum (ICD-O-3 C18-C20) between 1986 and 2006 in the Dutch population. In line with the results of our analysis, it is thus plausible that in a considerable number of CUP patients with a liver metastasis, the primary tumour origin is the colorectum. Furthermore, we have checked the potential of residual confounding by cigarette smoking status. Despite studying fewer cases in the categories of interest due to stratification based on sex and cigarette smoking status, the association between beef and processed meat consumption did not differ greatly between the strata (never, ex, current smokers) in women, thereby hinting that the potential of residual confounding is unlikely. We have also checked whether splitting the follow-up time had an influence on the association between meat consumption and CUP risk. We observed that the association between beef consumption and CUP risk was highest in the first 10 years of follow-up, whereas it attenuated in the last 10 years of follow-up. For processed meat consumption and CUP risk, no association was found in the first 10 years of follow-up, while there was a positive statistically significant association in the last 10 years of follow-up. An indication for these findings might be that there is a shorter latency period for beef consumption and a relatively longer latency period for processed meat consumption, or that it concerns a chance finding as there were fewer cases available due to splitting the follow-up time. Therefore, more studies would be needed to investigate such conclusions.

As briefly presented in the introduction, scientific evidence has already revealed associations between red meat intake and processed meat intake and the development of specific cancers, though the associations are less consistent concerning poultry and fish consumption and carcinogenesis [13, 17]. As we have demonstrated here, however, there does appear to be a discernible connection between the consumption of beef and processed meats and the development of CUP (Table 4).

### Strengths and limitations

Important strengths of this study are its prospective cohort design, large sample size of 1,208,52 participants, large number of incident CUP cases, and the detailed availability of exposure and confounder data. Moreover, completeness of record linkage with the NCR and PALGA for cancer follow-up was at least 96%, which minimizes selection bias [28]. Vital status follow-up was complete for almost 100% after 20.3 years. Details on incident CUP cases were obtained from the NCR and included specific information from both pathology reports and clinical reports [29]. In addition, we could access the pathology excerpts and thus check whether the cytological and/or histological confirmed cases had been correctly categorised in the data provided by the NCR. The NCR registry clerks applied uniform coding rules when entering data based on medical files.

There are certain limitations that should be acknowledged. Exposure data on meat consumption were only measured at baseline in 1986, so participants may have changed their dietary habits after having completed the questionnaire, which could result in bias due to misclassification. The questionnaire was tested, however, both for validity and reproducibility purposes and appeared to be representative for dietary habits over a period of at least 5 years [22, 23]. In addition, this potential bias should be non-differential between CUP cases and subcohort members.

### Conclusions

Beef and processed meat consumption appear to be positively associated with CUP risk in women. Similarly, a positive association was found between processed meat consumption and CUP risk in men, although it was not statistically significant. We found no associations between red meat (overall), poultry, or fish consumption and CUP risk.

**Supplementary Information** The online version contains supplementary material available at [https://doi.org/10.1007/s00394-021-02600-5](https://doi.org/10.1007/s00394-021-02600-5).
Acknowledgements We gratefully thank the participants and employees of the NLCS for their invaluable contributions. We thank the NCR and PALGA for providing extensive data on cancer follow-up.

Funding This work was supported by the Wereld Kanker Onderzoek Fonds (WCRF-NL), as part of the World Cancer Research Fund International grant program (grant number 2017/1628).

Availability of data and material The data sets generated and/or analysed during the current study are not publicly available, because the informed consent does not allow for that. However, anonymous data that are minimally required to replicate the outcomes of the study will be made available upon reasonable request and approval by the institutional review boards.

Declarations

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval The institutional review boards of The Netherlands Organization for Applied Scientific Research TNO (Zeist) and Maastricht University (Maastricht) approved the execution of the NLCS. Participants agreed to be included into the cohort and follow-up by returning the questionnaire they completed.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Loffler H, Kramer A (2016) Introduction. In: Kramer A, Loffler H (eds) Cancer of Unknown Primary. Springer, Switzerland
2. Schroten-Loef C, Verhoeven R, de Hingh I, van de Wouw A, van Laarhoven H, Lemmens V (2018) Unknown primary carcinoma in the Netherlands: decrease in incidence and survival times remain poor between 2000 and 2012. Eur J Cancer 101:77–86
3. Comprehensive Cancer Centre the Netherlands. 2020 Incidence primaire tumor onbekend. Integraal kankercentrum Nederland. https://iknl.nl/kankersoorten/primaire-tumor-onbekend/registratie/incidentie. Accessed 2 Nov 2020
4. Meijer L, De Peuter R, Van der Zwan JM, Loef C 2020 Primaire tumor onbekend. Integraal kankercentrum Nederland. https://www.iknl.nl/getmedia/ea952e4d-9af7-4d59-9003-805c855bcb0cd/Rapport_PrimaireTumorOnbekend_IKNL_2020.pdf. Accessed 2 Nov 2020
5. Pavlidis N, Pentheroudakis G (2012) Cancer of unknown primary site. Lancet 379(9824):1428–1435. https://doi.org/10.1016/S0140-6736(11)61178-1
6. Hemminki K (2016) Epidemiology risk factors and survival in cup: pointers to disease mechanisms.In: cancer of unknown primary. Springer, New York, pp 5–25
7. Rassy E, Pavlidis N (2020) Progress in refining the clinical management of cancer of unknown primary in the molecular era. J Nature Rev Clin Oncol 17(9):541–554
8. Fizazi K, Greco FA, Pavlidis N, Daugaard G, Oen K, Pentheroudakis G, EG Committee (2015) Cancers of unknown primary site ESMO clinical practice guidelines for diagnosis, treatment and follow up. Ann Oncol. https://doi.org/10.1093/annonc/mdv305
9. Hemminki K, Chen B, Melander O, Manjer J, Hallmans G, Hemminki A (2015) Smoking and body mass index as risk factors for subtypes of cancer of unknown primary. Int J Cancer 136(1):246–247. https://doi.org/10.1002/ijc.28969
10. Kaaks R, Sookthai D, Hemminki K, Kramer A, Boeving W, Wahfert E, Weienderpass E, Overvad K, Tjonneland A, Olsen A, Peeters PH, Bueno-de-Mesquita HB, Panico S, Pala V, Vineis P, Quirios JR, Ardanaz E, Sanchez MJ, Chirilae MD, Larraanaga N, Brennan P, Trichopolous D, Trichopolous A, Lagoi P, Hallmans G, Khaw KT, Key TJ, Riboli E, Canzian F (2014) Risk factors for cancers of unknown primary site: Results from the prospective EPIC cohort. Int J Cancer 135(10):2475–2481. https://doi.org/10.1002/ijc.28874
11. Vajdic C, Perez-Concha O, Dobbins T, Ward RL, Schaffer AL, van Leeuwen M, Rhee JJ, Laaksonen M, Craigen G, Pearson S-A (2019) Demographic, social and lifestyle risk factors for cancer registry-notified cancer of unknown primary site (CUP). Cancer Epidemiol 60:156–161
12. Hermans KE, van den Brandt PA, Loef C, Jansen RL, Schouten LJ (2021) Alcohol consumption, cigarette smoking and cancer of unknown primary (CUP) risk: results from the Netherlands Cohort Study. Int J Cancer. https://doi.org/10.1002/ijc.33328
13. Bouvard V, Loomis D, Guyton KZ, Grosse Y, Ghissassi FE, Benbrahim-Tallaa L, Guha N, Mattock H, Straif K, DJTLO Corpet (2015) Carcinogenicity of consumption of red and processed meat. Lancet Oncol 16(16):1599–1600
14. Ferguson L (2010) Meat and cancer. Meat Sci 84(2):308–313
15. Alimohar A, Al-Zenki S, Al-Hooti S, Zaghloul S, Sawaya W, Ahmed N, Kannan KF (2011) Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs) from grilled and smoked foods. Food Control 22(12):2028–2035
16. Daniel CR, Cross AJ, Graubard BI, Hollenbeck AR, Park Y, Sinha R (2011) Prospective investigation of poultry and fish intake in relation to cancer risk. Cancer Prev Res 4(11):1903–1911
17. World Cancer Research Fund/American Institute for Cancer Research. 2018 Meat, fish and dairy products and the risk of cancer. https://www.wcrf.org/dietandcancer. Accessed 2 Nov 2020
18. Goldbohm RA, van den Brandt PA, van’t Brantsveer HAP, Al M, SturmsHermus FRJ (1990) A large-scale prospective cohort study on diet and cancer in The Netherlands. J Clin Epidemiol 43(3):285–295
19. Van den Brandt PA, Schouten LJ, Goldbohm RA, Dorant E, Hunen PM (1990) Development of a record linkage protocol for use in the Dutch Cancer Registry for epidemiological research. Int J Epidemiol 19(3):553–558
20. Goldbohm RA, van den Brandt PA, van’t Brantsveer HAP, Al M, SturmsHermus FRJ (1994) A prospective cohort study on the relation between meat consumption and the risk of colon cancer. Cancer Res 54(3):718–723
21. Van den Brandt P (2019) Red meat, processed meat, and other dietary protein sources and risk of overall and cause-specific mortality in The Netherlands cohort study. Eur J Epidemiol 34(4):351–369
22. Goldbohm RA, van den Brandt PA, van’t Brantsveer HAP, Al M, SturmsHermus FRJ (1994) Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. Eur J Clin Nutr 48(4):253–265
23. Goldbohm RA, van den Brandt PA, van’t BrantsVeer HAP, Al M, SturmansHermus FRJ (1995) Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. Eur J Clin Nutr 49(6):420–429
24. Barlow WE (1994) Robust variance estimation for the case-cohort design. Biometrics 50(4):1064–1072
25. Lin DY, Wei L-J (1989) The robust inference for the Cox proportional hazards model. J Am Stat Assoc 84(408):1074–1078
26. World Cancer Research Fund/American Institute for Cancer Research 2018 Diet, Nutrition, Physical Activity and Cancer: a Global Perspective. Continuous Update Project Expert Report 2018.
27. Riihimäki M, Hemminki A, Sundquist J, Hemminki K (2016) Patterns of metastasis in colon and rectal cancer. J Sci Rep 6(1):1–9
28. Goldbohm RA, Van den Brandt PA, Dorant E (1994) Estimation of the coverage of Dutch municipalities by cancer registries and PALGA based on hospital discharge data. Tijdschr Soc Gezondheidsz 72:80–84
29. Van der Sanden G, Coebergh J-W, Schouten L, Visser O, Van Leeuwen F (1995) Cancer incidence in the Netherlands in 1989 and 1990: First results of the nationwide Netherlands Cancer Registry. Eur J Cancer 31(11):1822–1829