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Red and processed meat intake and cancer risk: Results from the prospective NutriNet-Santé cohort study

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Cancer Epidemiology

The International Agency for Research on Cancer (WHO-IARC) classified red meat and processed meat as probably carcinogenic and carcinogenic for humans, respectively. These conclusions were mainly based on studies concerning colorectal cancer, but scientific evidence is still limited for other cancer locations. In this study, we investigated the prospective associations between red and processed meat intakes and overall, breast, and prostate cancer risk. This prospective study included 61,476 men and women of the French NutriNet-Santé cohort (2009–2015) aged ≥35 y and who completed at least three 24 hrs dietary records during the first year of follow-up. The risk of developing cancer was compared across sex-specific quintiles of red and processed meat intakes by multivariable Cox models. 1,609 first primary incident cancer cases were diagnosed during follow-up, among which 544 breast cancers and 222 prostate cancers. Red meat intake was associated with increased risk of overall cancers [$HR_{Q5vs.Q1}$=1.31 (1.10–1.55), p_{trend} = 0.01) and breast cancer ($HR_{Q5vs.Q1}$ = 1.83 (1.33–2.51), p_{trend} = 0.002]. The latter association was observed in both premenopausal [$HR_{Q5vs.Q1}$=2.04 (1.03–4.06)] and postmenopausal women [$HR_{Q5vs.Q1}$=1.79 (1.26-2.55)]. No association was observed between red meat intake and prostate cancer risk. Processed meat intake was relatively low in this study (cut-offs for the 5th quintile = 46 g/d in men and 29 g/d in women) and was not associated with overall, breast or prostate cancer risk. This large cohort study suggested that red meat may be involved carcinogenesis at several cancer locations (other than colon-rectum), in particular breast cancer. These results are consistent with mechanistic evidence from experimental studies.

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The International Agency for Research on Cancer (WHO-IARC) recently classified consumption of processed meat as "carcinogenic to humans" and consumption of red meat as "probably carcinogenic to humans."[1] The World Cancer Research Fund and the American Institute for Cancer Research (WCRF/AICR) recommends consuming <500 g/week of red meat and <50 g/d of processed meat.[2] These conclusions were mainly based on findings concerning colorectal cancer, for which the weight of evidence is considered as convincing.[3,4] Indeed, experimental studies showed that several components of red and/or processed meat act locally on the colorectal mucosa to promote carcinogenesis. Potential carcinogens include heme iron, nitrates and nitrites and mutagenic compounds such as neoformed products generated in red meats and processed meat (heterocyclic amines, polycyclic aromatic hydrocarbons, N-nitroso compounds.[3,5,6] However, these procarcinogens may also be involved in more systemic mechanisms,[7–10] suggesting that red and processed meat may impact cancer risk for cancer locations other than colon-rectum.

Despite these mechanistic hypotheses, epidemiological evidence regarding red/processed meat and cancer risk is limited for other cancer locations, and notably for breast and prostate cancers, which are the two main cancer sites in many Western countries.[11,12] In a previous study performed in the

What's new?

Red meat contains multiple substances that are potentially carcinogenic, including nitrates, nitrites, and heterocyclic amines. Its consumption, presumably owing to the presence of these substances, is associated with carcinogenic processes primarily in the colorectal mucosa. The present study shows, however, that red meat intake is also associated with increased risk of cancer overall, as well as with increased breast cancer risk specifically. Breast cancer risk was elevated for both premenopausal and postmenopausal women. The findings indicate that red meat intake affects more than the colorectal mucosa and that its restriction could be important in preventing tumors at other sites.

SU.VI.MAX cohort,[13] we observed that processed meat intake was associated with increased breast cancer risk. This result is consistent with two recent meta-analyses suggesting positive associations with breast cancer risk.[14,15] Since the publication of these meta-analyses, two prospective cohort studies were published. Inoue-Cho *et al.*[16] observed an increased risk of breast cancer in post-menopausal women with high consumption of red or processed meat; and Bertrand *et al.*[17] showed increased breast density in pre-menopausal women associated with high consumption of red meat. In 2014, the World Cancer Research Fund and the American Institute for Cancer Research (WCRF/AICR) observed null results for their meta-analyses of the associations between red and processed meat and prostate cancer risk,[18] consistent with a meta-analysis published in 2015.[19] In contrast, in a pooled analysis of 15 cohort studies published in 2016, Wu *et al.*[20] observed a positive association between red and processed meat and risk of advanced prostate cancer. Thus, the weight of evidence is still considered as "limited" regarding red and processed meat and cancer risk for non-colorectal locations.[3,18,21,22] No consensus has been reached so far and additional prospective studies are needed to more thoroughly elucidate the relationship between red and processed meat intakes and breast or prostate cancer risk.

The objective of this prospective study was to investigate the associations between red meat and processed meat intakes and overall, breast and prostate cancer risk, in a large cohort of French adults with accurate and up-to-date dietary intake data.

Methods

Study population

The NutriNet-Santé study is an ongoing web-based cohort launched in 2009 in France with the objective to study the associations between nutrition and health as well as the determinants of dietary behaviors and nutritional status. This cohort has been previously described in details.[23] Participants aged over 18 years with access to the Internet are continuously recruited since May 2009 among the general population by means of vast multimedia campaigns. All questionnaires are completed online using a dedicated website (www.etude-nutrinet-sante.fr). The NutriNet-Santé study is conducted according to the Declaration of Helsinki guidelines and was approved by the Institutional Review Board of the French Institute for Health and Medical Research (IRB Inserm n°0000388FWA00005831) and the "Commission Nationale de l'Informatique et des Libertés" (CNIL n°908450/n°909216). Electronic informed consent is obtained from each participant (EudraCT no. 2013–000929-31).

Data collection

Dietary data. Dietary intakes were assessed every 6 months through a series of three non-consecutive validated web-based 24 hrs-dietary records, randomly assigned over a 2-week period (2 weekdays and 1 weekend day).[24–26] Participants used a dedicated interface of the study website to declare all foods and beverages consumed during a 24 hrs-period: three main meals (breakfast, lunch, dinner) or any other eating occasion. Portion sizes were estimated using validated photographs.[27] Mean daily energy, alcohol and nutrient intakes were estimated using a published French food composition table (>3,300 items).[28] Amounts consumed from composite dishes were estimated using French recipes validated by food and nutrition professionals. Dietary underreporting was identified on the basis of the method proposed by Black.[29] Red meat intake was defined as fresh, minced and frozen beef, veal, pork, and lamb. Processed meat intake was defined as mostly pork and beef preserved by methods other than freezing, such as salting, smoking, marinating, air-drying or heating and included ham, bacon, sausages, blood sausages, liver pâté, salami, mortadella, tinned meat and others.

Covariates. At inclusion, participants fulfilled a set of five questionnaires related to socio-demographic and lifestyle characteristics[30] (*e.g.*, sex, date of birth, educational level, smoking status, number of children), anthropometrics[31,32] (*e.g.*, height and weight), dietary intakes (see above), physical activity (validated IPAQ questionnaire)[33] and health status (*e.g.*, personal and family history of diseases, medication use including hormonal treatment for menopause and oral contraception, menopausal status).

Case ascertainment

Participants self-declared health events through the yearly health status questionnaire, through a specific check-up questionnaire for health events (every 3 months) or at any time through a specific interface on the study website. Following this declaration, participants were invited to send their

medical records (diagnosis, hospitalization, *etc.*) and, if necessary, the study physicians contacted the participants' treating physician or the medical structures to collect additional information. Then, data were reviewed by an independent physician expert committee for the validation of major health events. Cancer cases were classified using the International Chronic Diseases Classification, 10th Revision, Clinical Modification (ICD-10).[34] In this study, all first primary cancers diagnosed between the inclusion and August 2015 were considered as cases (except basal cell skin carcinoma, which was not considered as cancer).

Statistical analyses

So far, 96,716 subjects without cancer at baseline provided at least three valid 24 hrs-dietary records during their first year of follow-up. Participants aged <35 y ($n = 32{,}882$) were excluded because of a very low susceptibility to develop cancer and so were subjects with a null follow-up ($n = 2{,}358$). Thus, 61,476 subjects were included in the analyses.

Estimated red and processed meat and other dietary intakes were based on the average intake for each subject across all 24 hrs-dietary records available in their first year of follow-up. For all covariates except physical activity, $\leq 5\%$ of values were missing and were imputed to the modal value. For physical activity (13% of missing values), a "missing class" was introduced into the models.

Baseline characteristics of participants were compared across sex-specific quintiles of red and processed meat intake using χ^2 tests or Fisher tests wherever appropriate. We estimated hazard ratios (HR) and 95% confidence intervals (CI) using Cox proportional hazards models, with age as the primary time variable, to characterize the association between sex-specific quintiles of red meat, processed meat and total red and processed meat intake and incidence of overall, breast or prostate cancer risk (the two main cancer locations in the cohort). We confirmed that the assumptions of proportionality were satisfied through examination of the log-log (survival) versus log-time plots. Tests for linear trend were performed using the ordinal score on sex-specific quintiles of intake. Participants contributed person-time until the date of cancer diagnosis, the date of last completed questionnaire, the date of death, or August 31, 2015, whichever occurred first. For cancer site specific analysis, women who reported a cancer other than breast cancer and men who reported a cancer other than prostate cancer during the study period were censored at the date of diagnosis. Analyses were performed according to menopausal status for breast cancer analyses. For these analyses, women contributed person-time in the Cox model until their date of menopause for premenopausal breast cancer analysis or from their date of menopause for postmenopausal breast cancer analysis. Additionally, models restricted to invasive breast cancer cases (excluding *in situ* cases) were tested.

Models were adjusted for age (time-scale), sex (for overall cancers only), BMI (kg/m^2, continuous), height (cm, continuous), physical activity (high, moderate, low, computed following IPAQ recommendations[35]), smoking status (never smokers, former smokers, current smokers), number of 24 hrs-dietary records (continuous), fruits and vegetables intake (g/d, continuous), total lipids intake (g/d, continuous), alcohol intake (g/d, continuous), energy intake (without alcohol, g/d, continuous), family history of cancer (yes/no) and educational level (<high-school degree, <2 years after high-school degree, ≥2 years after high-school degree). Red and processed meat models were mutually adjusted for processed meat and red meat intakes, respectively. For breast cancer analyses, additional adjustments were performed for the number of biological children (continuous), menopausal status at baseline (yes/no), hormonal treatment for menopause at baseline (only for postmenopausal analyses, yes/no) and oral contraception use at baseline (only for premenopausal analyses, yes/no). Since antioxidants may partly counteract lipid peroxidation by heme iron from red and processed meat (*i.e.*, one of the hypothesized mechanisms involved in their potentially procarcingenic effect)[1], we have tested for a potential interaction between fruit and vegetable intake (as a proxy for antioxidant exposure, according to sex-specific median intake) and red and processed meat intake by introducing the product of the two variables into Cox models for each cancer location. Stratified analyses were performed when appropriate (*i.e.*, p-interaction < 0.1).

All tests were two-sided, and $p < 0.05$ was considered statistically significant. SAS version 9.4 (SAS Institute) was used for the analyses.

Results

Between May 2009 and August 2015 (median follow-up time: 4.1 year; 229,835 person-years), 1,609 incident cancer cases were diagnosed, among which 544 breast cancers (169 premenopausal and 375 postmenopausal; 71.6% ER+/PR+, 13.5% ER–/PR–, 14.6% ER+/PR–, 0.3% ER-/PR+; 80.4% invasive and 19.6% *in situ*), 222 prostate cancers (88,46% Gleason score < 7, 11,54% Gleason score ≥7) and 843 other cancers (169 skin (other than basal cell carcinoma), 120 colorectal, 64 lymphomas, 63 lung, 39 thyroid, 38 cervix, 38 bladder, 37 uterus, 35 leukemia, 30 kidney and 210 others).

Mean age at diagnosis was 51.68 y ± 10.14 and mean baseline-to-diagnosis time was 2.43 y ± 1.60. Mean number of 24 hrs dietary records per subject over their first year of follow-up was 4.53 ± 1.61.

Characteristics of the participants according to quintiles of total red and processed meat intakes are described in Table 1. Mean daily red meat intake was 42.9 ± 39.0 g/d (0.4 ± 1.9 g/d in the first quintile, 102.3 ± 33.7 g/d in the fifth quintile). Mean daily processed meat intake was 19.1 ± 23.8 g/d (0 g/d in the first quintile, 56.0 ± 25.9 g/d in the fifth quintile; data not tabulated). Subjects with higher total red and processed meat intake were more likely to be younger, to have a higher body mass index, to smoke, to have higher energy, lipid and alcohol intakes and lower fruit

Table 1. Baseline characteristics of study participants ($n = 61,476$) according to sex-specific quintiles of red and processed meat intake, NutriNet-Santé cohort, France, 2009–2016[1]

	Quintile 1 ($n = 12,292$)	Quintile 2 ($n = 12,298$)	Quintile 3 ($n = 12,303$)	Quintile 4 ($n = 12,287$)	Quintile 5 ($n = 12,296$)
Age, y	51.7 +/− 10.2	52 +/− 10.3	52.2 +/− 10.2	51.9 +/− 10.1	50.6 +/− 9.8
Sex					
Men	3,107 (25.28)	3,111 (25.29)	3,110 (25.28)	3,108 (25.30)	3,110 (25.29)
Women	9,185(74.72)	9,187(74.71)	9,193(74.72)	9,179(74.70)	9,186(74.71)
Height, cm	166.5 +/− 8.2	166.4 +/− 8.1	166.4 +/− 8.2	166.6 +/− 8.2	167.1 +/− 8.3
Body mass index, kg/m^2	23.4 +/− 4.2	23.9 +/− 4.2	24.3 +/− 4.4	24.9 +/− 4.6	25.7 +/− 5.2
Family history of cancer[2], yes	5,429 (44.2)	5,557 (45.2)	5,627 (45.7)	5,570 (45.3)	5,465 (44.4)
Number of children, *n*	1.7 +/− 1.2	1.8 +/− 1.2	1.9 +/− 1.1	1.9 +/− 1.2	1.9 +/− 1.1
Higher education					
No	2,718 (22.1)	2,806 (22.8)	3,034 (24.7)	3,316 (27.0)	3,543 (28.8)
Yes, < 2 years	1,885 (15.3)	1,876 (15.3)	1,785 (14.5)	1,955 (15.9)	2,042 (16.6)
Yes, ≥ 2 years	7,689 (62.6)	7,616 (61.9)	7,484 (60.8)	7,016 (57.1)	6,711 (54.6)
Smoking status					
Current	1,476 (12.0)	1,476 (12.0)	1,542 (12.5)	1,777 (14.5)	2,116 (17.2)
Former	5,063 (41.2)	5,046 (41.0)	5,000 (40.6)	5,042 (41.0)	5,109 (41.6)
Never	5,753 (46.8)	5,776 (47.0)	5,761 (46.8)	5,468 (44.5)	5,071 (41.2)
IPAQ Physical activity level[3]					
High	4,292 (34.9)	4,056 (33)	3,881 (31.5)	3,802 (30.9)	3,628 (29.5)
Moderate	4,524 (36.8)	4,460 (36.3)	4,413 (35.9)	4,290 (34.9)	4,001 (32.5)
Low	2,049 (16.7)	2,316 (18.8)	2,452 (19.9)	2,600 (21.2)	2,870 (23.3)
Processed meat intake, g/d	3.8 +/− 6.1	11.6 +/− 12.2	17.3 +/− 16.7	24.2 +/− 21.6	38.6 +/− 34.6
Red meat intake, g/d	3.6 +/− 6.7	22.3 +/− 13.8	38.5 +/− 17.9	56.2 +/− 23.0	93.9 +/− 42.0
Fruits and vegetables intake, g/d	496.9 +/− 257.7	458.7 +/− 211.6	442.4 +/− 203.0	431.6 +/− 198.8	411.4 +/− 203.5
Energy intake, kcal/d	1,720 +/− 443.9	1,769.1 +/− 425.2	1,805 +/− 433.3	1,844.5 +/− 436.0	1,962.3 +/− 492.1
Total lipid intake, g/d	73 +/− 24.5	76.4 +/− 23.1	79.3 +/− 23.4	82.6 +/− 24.2	90.9 +/− 27.8
Alcohol intake, g/d	6.3 +/− 10.1	7.8 +/− 11.0	8.7 +/− 11.7	9.9 +/− 13.3	11.9 +/− 15.9
Oral contraception, yes	1,021 (11.1)	1,118 (12.2)	1,112 (12.1)	1,176 (12.8)	1,299 (14.1)
Hormonal treatment for menopause, yes	750 (8.2)	830 (9.0)	882 (9.6)	827 (9.0)	699 (7.6)
Menopausal, yes	4,557 (49.6)	4,546 (49.5)	4,698 (51.1)	4,537 (49.4)	4,056 (44.2)

[1]Values are means ±SDs or *n* (%). Cut-offs for quintiles of red and processed meat intake were 32.00; 59.82; 86.81 and 122.14 g/d in men and 18.21; 39.73; 60.00 and 87.68 g/d in women.
[2]Among first-degree relatives.
[3]Missing for 7,842 (12.76%) subjects.

and vegetable intake, to have a lower educational level and to be less physically active.

Associations between red and processed meat intakes and overall, breast and prostate cancer risk are presented in Table 2. Red meat intake was associated with increased overall cancer risk (HR $_{Q5vs.Q1} = 1.31$; 95% CI 1.10, 1.55; $p_{trend} = 0.01$) and increased breast cancer risk (HR $_{Q5.Q1} = 1.83$; 95% CI 1.33, 2.51; $p_{trend} = 0.002$), but not with prostate cancer risk ($p_{trend} = 0.9$). This association between red meat intake and increased breast cancer risk was observed in both premenopausal ($HR_{Q5vs.Q1} = 2.04$ (1.03–4.06)) and postmenopausal women [$HR_{Q5vs.Q1} = 1.79$ (1.26–2.55); Table 3], and was similarly observed when analyses excluded cases diagnosed during their first year of follow-up [413 cases/40,892 non-cases included; $HR_{Q5vs.Q1} = 1.82$ (1.27, 2.62)] or when analyses were restricted to invasive breast cancers [470 cases/45,386 non-cases; $HR_{Q5vs.Q1} = 1.78$ (1.26, 2.50); data not tabulated. Results with and without BMI adjustment were very similar for overall, breast and prostate cancers models (without BMI adjustment in Supporting Information Table 2). No association was detected for processed meat intake ($p_{trend} = 0.5$, 0.4 and 0.3 for overall, breast and prostate cancers, respectively,

Table 2. Associations between quintiles of red and processed meat intake and overall, breast, and prostate cancer risk, from multivariable Cox proportional hazard models, NutriNet-Santé cohort, France, 2009–2016 ($n = 61,476$)[1]

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*p*-trend
Red meat						
All cancers						0.01
N for cases/non-cases	233/12,101	359/11,898	307/12,001	358/11,876	352/11,991	
Multivariable HR (95%CI)	1	1.24 (1.05, 1.47)	1.06 (0.89, 1.26)	1.22 (1.03, 1.45)	1.31 (1.10, 1.55)	
Breast cancer						0.002
N for cases/non-cases	59/9,160	124/9,030	114/9,076	123/9,010	124/9,110	
Multivariable HR (95%CI)	1	1.68 (1.23, 2.31)	1.58 (1.14, 2.17)	1.70 (1.24, 2.34)	1.83 (1.33, 2.51)	
Prostate cancer						0.9
N for cases/non-cases	28/3,087	66/3,037	33/3,085	54/3,047	41/3,068	
Multivariable HR (95%CI)	1	1.70 (1.09, 2. 68)	0.87 (0.52, 1.45)	1.38 (0.86, 2.20)	1.28 (0.78, 2.11)	
Processed meat						
All cancers						0.5
N for cases/non-cases	403/17,148	221/6,830	350/1,1929	351/11,949	284/12,011	
Multivariable HR (95%CI)	1	1.08 (0.91, 1.28)	1.03 (0.88, 1.19)	1.05 (0.90, 1.22)	0.93 (0.79, 1.10)	
Breast cancer						0.4
N for cases/non-cases	133/13,809	63/4,380	113/9,055	134/9,057	101/9,085	
Multivariable HR (95%CI)	1	1.19 (0.88, 1.62)	1.08 (0.83, 1.39)	1.28 (1.00, 1.64)	1.05 (0.80, 1.38)	
Prostate cancer						0.3
N for cases/non-cases	37/3,572	42/2,566	57/3,054	45/3,064	41/3,068	
Multivariable HR (95%CI)	1	1.21 (0.77, 1.91)	1.39 (0.91, 2.13)	1.17 (0.74, 1.84)	1.35 (0.84, 2.20)	
Red and processed meat						
All cancers						0.3
N for cases/non-cases	266/12,026	339/11,959	342/11,961	344/11,943	318/11,978	
Multivariable HR (95%CI)	1	1.11 (0.94, 1.30)	1.08 (0.91, 1.27)	1.10 (0.93, 1.30)	1.12 (0.94, 1.33)	
Breast cancer						0.05
N for cases/non-cases	80/9,105	101/9,086	128/9,065	126/9,053	109/9,077	
Multivariable HR (95%CI)	1	1.10 (0.82, 1.49)	1.35 (1.02, 1.81)	1.36 (1.02, 1.81)	1.26 (0.93, 1.71)	
Prostate cancer						0.8
N for cases/non-cases	37/3,070	48/3,063	54/3,056	42/3,066	41/3,069	
Multivariable HR (95%CI)	1	1.07 (0.69, 1.65)	1.21 (0.79, 1.85)	0.93 (0.59, 1.48)	1.17 (0.72, 1.89)	

Sex-specific cut-offs for quintiles of red meat intake were 12.59; 37.14; 57.15 and 86.75 g/d in men and 0.14; 24.67; 42.15 and 65.71 g/d in women.
Sex-specific cut-offs for quintiles of processed meat intake were 0.20; 11.61; 25.45 and 45.86 g/d in men and 0.06; 5.36; 14.64 and 29.00 g/d in women.
Sex-specific cut-offs for quintiles of red and processed meat intake were 32.00; 59.82; 86.81 and 122.14 g/d in men and 18.21; 39.73; 60.00 and 87.68 g/d in women.
CI, confidence interval, HR, Hazard ratio.
[1]Multivariable models were adjusted for age (timescale), sex, energy intake without alcohol, number of 24 hrs-dietary records, smoking status, educational level, physical activity, height, BMI, alcohol intake, family history of cancers, lipids intake, fruits, vegetables, menopausal status and number of children (breast cancer models), red meat intake (where processed meat was analyzed) and processed meat intake (where red meat was analyzed).

Table 2). No association was statistically significant for red or processed meat intake with colorectal or with lung cancers or with lymphomas (Supporting Information Table 1). No interaction was detected between red or processed meat intake and fruit and vegetable or individual antioxidant intakes (vitamins C, E, beta-carotene and selenium) regarding overall and site-specific cancer risk (all $p > 0.05$, data not shown).

Discussion

In this large prospective cohort, red meat intake was significantly associated with increased overall and breast cancer risks. No association was observed for prostate cancer. Processed meat intake was not associated with cancer risk in this study.

For red meat, our result of a direct association with breast cancer risk is consistent with two recent meta-analyses: Guo

Table 3. Associations between quintiles of red and processed meat intake and breast cancer risk according to menopausal status from multivariable Cox proportional hazards models, NutriNet-Santé cohort, France, 2009–2016 ($n = 46,474$)[1]

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*p*-trend
Red meat						
Pre-menopausal breast cancer						0.4
N for cases/non-cases	12/4,732	50/4,502	36/4,618	43/4,608	28/4,622	
Multivariable HR (95%CI)	1	3.36 (1.77, 6.38)	2.37 (1.22, 4.60)	2.91 (1.52, 5.57)	2.04 (1.03, 4.06)	
Post-menopausal breast cancer						0.002
N for cases/non-cases	48/5,347	73/5,307	81/5,308	78/5,319	95/5,281	
Multivariable HR (95%CI)	1	1.28 (0.88, 1.86)	1.46 (1.02, 2.09)	1.40 (0.97, 2.01)	1.79 (1.26, 2.55)	
Processed meat						
Pre-menopausal breast cancer						0.5
N for cases/non-cases	32/6,591	28/2,645	32/4,619	40/4,614	37/4,613	
Multivariable HR (95%CI)	1	1.62 (0.96, 2. 73)	1.09 (0.66, 1.80)	1.34 (0.83, 2.17)	1.30 (0.79, 2.15)	
Post-menopausal breast cancer						0.7
N for cases/non-cases	101/8,309	36/2,327	79/5,299	93/5,306	66/5,321	
Multivariable HR (95%CI)	1	1.08 (0.73, 1.60)	1.07 (0.79, 1.44)	1.28 (0.95, 1.72)	0.95 (0.69, 1.32)	
Red and processed meat						
Pre-menopausal breast cancer						0.8
N for cases/non-cases	23/4,609	36/4,632	41/4,612	40/4,608	29/4,621	
Multivariable HR (95%CI)	1	1.29 (0.76, 2.19)	1.40 (0.83, 2.36)	1.40 (0.83, 2.37)	1.05 (0.59, 1.86)	
Post-menopausal breast cancer						0.02
N for cases/non-cases	57/5,331	66/5,304	80/5,325	88/5,299	84/5,303	
Multivariable HR (95%CI)	1	1.06 (0.74, 1.52)	1.26 (0. 90, 1.77)	1.40 (0.99, 1.96)	1.41 (0.99, 2.01)	

In premenopausal women: cut-offs for quintiles of red meat intake were 0.29; 24.00; 42.14; 67.7 g/d; cut-offs for quintiles of processed meat intake were 0.11; 6.79; 16.43; 31.89 g/d; cut-offs for quintiles of red and processed meat intake were 18.57; 40.40; 61.79; 91.16 g/d.
In postmenopausal women: cut-offs for quintiles of red meat intake were 2.68; 25.37; 42.68; 65.00 g/d; cut-offs for quintiles of processed meat intake were 0.06; 5.14; 14.29; 27.26 g/d; cut-offs for quintiles of red and processed meat intake were 18.21; 39.29; 58.79; 85.06 g/d..
CI: confidence interval, HR: Hazard ratio.
[1]Multivariable models were adjusted for age (timescale), energy intake without alcohol, number of 24 hrs-dietary records, smoking status, educational level, physical activity, height, BMI, alcohol intake, family history of cancers, lipids intake, fruits, vegetables, hormone replacement therapy (for postmenopausal group), number of children, contraception (for premenopausal group), red meat intake (where processed meat was analyzed) and processed meat intake (where red meat was analyzed).

et al.[14] based on 14 cohort studies for red meat and 12 cohort studies for processed meat, and Wu *et al.*[15] based on 12 cohort studies for red meat and 15 cohort studies for processed meat, both showing positive associations with breast cancer risk. The two prospective studies published after this meta-analysis also suggest direct associations between red meat intake and post-menopausal breast cancer risk in the NIH-AARP cohort[16] and increased breast density.[17] In a previous study performed on the SU.VI.MAX cohort, we did not observe statistically significant relationships between red meat and breast cancer risk. However, red meat intakes in women of the SU.VI.MAX cohort were relatively low (fourth quartile <500 g/week), while they were higher in the present NutriNet-Santé cohort, where 19.60% exceeded 500 g of red meat per week. In the French general population, about one out of four adults consume >500 g/week of red meat.[3] In Europe the median range of daily red meat intake is 24–57 g/day,[36] while mean intake is about 53 g/d in the U.S.[37]

Regarding prostate cancer, our null result is consistent with two large and recent meta-analyses of prospective studies, performed by the WCRF/AICR in 2014[18] and Blysma *et al.* in 2015.[19] In a pooled analysis of 15 cohort studies, Wu *et al.*[20] did not observe any association between red meat intake and overall prostate cancer risk, but showed a modest positive association for tumors identified as advanced stage at diagnosis. In our study, our results did not differ according to Gleason score (< or ≥7) [data not shown]. However, statistical power was limited for this sub-analysis. In the WCRF/AICR meta-analyses, the summary RR were not statistically significant for the different prostate cancer subtypes, (RR per 100 g/d = 0.99 (0.89, 1.11) for advanced/high grade and 1.19 (0.88, 1.59) for fatal cases).[18]

The small number of cancer cases other than breast and prostate locations did not allow us to have enough statistical analysis to conclude for these locations. However, the pro-carcinogenic effect of high red meat intake on colorectal carcinogenesis has been well established in several national and

international collective expert evaluations.[1,3,4] In 2012, the WCRF/AICR also judged the direct association between red meat intake and pancreatic cancer risk as "suggestive". Along with the positive association observed for breast cancer, these may contribute to explain the positive association observed in the present study between red meat intake and overall cancer risk. It is also possible that the lack of association with processed meat might be a chance finding or could change with longer follow-up.

While several studies suggested direct associations between processed meat intake and colorectal,[1,3,4] breast,[13,14,16] stomach,[38] or pancreatic[39] cancer risk, no association was detected in the present study. This may be explained by the fact that processed meat intakes were too low to properly investigate any adverse effect. Indeed, the cut-off for quintile 5 of processed meat intake was 45.9 g/d for men and 29.0 g/d for women, that is, lower than the 50 g/d upper dose recommended by the WCRF/AICR for colorectal cancer prevention.[4] In the French general population, more than one out of four adults consume at least 50 g of processed meat per Day.[3] In Europe the median range of daily processed meat intake is 5–49 g/Day,[36] while mean intake is about 18 g/d in the U.S.[37]

Our epidemiological findings are supported by mechanistic data. Red and processed meat contain pro-carcinogenic components, such as heterocyclic aromatic amines (HAA), polycyclic aromatic hydrocarbons (PAHs) resulting from meat processing or preparation (such as cooking at high-temperature), nitrites (used as additives) and induces N-nitroso compounds (NOCs) formation in the digestive tract.[40–44] These chemicals may exert a pro-carcinogenic effect through direct DNA damage and have been associated with mammary tumor development in animal[7,9,41] and human[8,10,45] studies.[13,46,47] Most importantly, red meat contains high levels of heme iron, which may contribute to initiate carcinogenesis via several mechanisms, including the production of genotoxic free radicals, NOCs or through lipid peroxidation.[5,48–50]

Strengths of this study include its prospective design, its large sample size, and the assessment of usual dietary intakes using repeated 24 hrs-dietary records based on a recent food composition database with a large choice of items (>3,300). These repeated 24 hrs-dietary records allowed a better insight into the food products consumed compared to food frequency questionnaires with more aggregated food items. However, some limitations should be acknowledged. First, caution is needed regarding the extrapolation of these results since this study included volunteers involved in a long-term cohort study investigating the association between nutrition and health, with overall more health-conscious behaviors and higher professional and/or educational level compared to the general French population. Thus, unhealthy dietary behaviors may have been underrepresented in this study, which may have weakened the observed associations and may have prevented us from observing significant associations for processed meat. Second, although the number of overall cancer cases was reasonably large, the number of cancers at any given site was more restricted, which did not allow us to investigate more cancers sites and receptor types for breast cancer. Finally, the observed relationships could be partly affected by unmeasured or residual confounding. However, main potential confounders have been accounted for in this study; thus, it is unlikely that residual confounding entirely explains the observed associations.

In conclusion, this prospective cohort study brings new contribution into the role of red and processed meat intake as cancer risk factors. We observed that red meat intake was associated with increased overall and breast cancer risk, in line with mechanistic hypotheses from experimental studies. If confirmed, these findings suggest that limiting red meat intake may not only be beneficial for colorectal cancer, but also for the prevention of other tumor locations such as breast cancer.

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