Accepted Manuscript

Title: Meat intake, methods and degrees of cooking and breast cancer risk in the MCC-Spain study

Authors: Elena Boldo, Adela Castelló, Nuria Aragonés, Pilar Amiano, Beatriz Pérez-Gómez, Gemma Castaño-Vinyals, Vicente Martín, Marcela Guevara, Carmen Urtiaga, Trinidad Dierssen-Sotos, Guillermo Fernández-Tardón, Victor Moreno, Marta Solans, Rosanna Peiró, Rocio Capelo, Inés Gómez-Acebo, Jesús Castilla, Antonio José Molina, Xavier Castells, Jose M. Altzibar, Virginia Lope-Carvajal, Manolis Kogevinas, Isabelle Romieu, Marina Pollán, on behalf of MCC-Spain researchers

PII: S0378-5122(17)30877-0
DOI: https://doi.org/10.1016/j.maturitas.2018.01.020
Reference: MAT 6955
To appear in: Maturitas
Received date: 14-9-2017
Revised date: 14-11-2017
Accepted date: 20-1-2018

Please cite this article as: Boldo Elena, Castelló Adela, Aragonés Nuria, Amiano Pilar, Pérez-Gómez Beatriz, Castaño-Vinyals Gemma, Martín Vicente, Guevara Marcela, Urtiaga Carmen, Dierssen-Sotos Trinidad, Fernández-Tardón Guillermo, Moreno Victor, Solans Marta, Peiró Rosanna, Capelo Rocio, Gómez-Acebo Inés, Castilla Jesús, Molina Antonio José, Castells Xavier, Altzibar Jose M, Lope-Carvajal Virginia, Kogevinas Manolis, Romieu Isabelle, Pollán Marina. Meat intake, methods and degrees of cooking and breast cancer risk in the MCC-Spain study. Maturitas https://doi.org/10.1016/j.maturitas.2018.01.020

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Meat intake, methods and degrees of cooking and breast cancer risk in the MCC-Spain study

Elena Boldo\textsuperscript{a,b,c}, Adela Castelló\textsuperscript{a,b,c}, Nuria Aragonés\textsuperscript{a,b,c}, Pilar Amiano\textsuperscript{b,d}, Beatriz Pérez-Gómez\textsuperscript{a,b,c}, Gemma Castaño-Vinyals\textsuperscript{b,e,f,g}, Vicente Martín\textsuperscript{b,h}, Marcela Guevara\textsuperscript{b,i}, Carmen Urtiaga\textsuperscript{d}, Trinidad Dierssen-Sotos\textsuperscript{b,i}, Guillermo Fernández-Tardón\textsuperscript{b,k}, Victor Moreno\textsuperscript{b,l,m}, Marta Solans\textsuperscript{b,n,o}, Rosanna Peiró\textsuperscript{b,p}, Rocio Capelo\textsuperscript{b,q}, Inés Gómez-Acebo\textsuperscript{b,i}, Jesús Castilla\textsuperscript{b,j}, Antonio José Molina\textsuperscript{b,h}, Xavier Castells\textsuperscript{r,s,t}, Jone M. Altzibar\textsuperscript{b,u}, Virginia Lope-Carvajal\textsuperscript{b,c}, Manolis Kogevinas\textsuperscript{b,e,f,g}, Isabelle Romieu\textsuperscript{v}, Marina Pollán\textsuperscript{a,b,c} on behalf of MCC-Spain researchers.

\textsuperscript{a} Cancer Epidemiology Unit, National Centre for Epidemiology, Carlos III Institute of Health, Avenida Monforte de Lemos 5, 28029 Madrid, Spain

\textsuperscript{b} Consortium for Biomedical Research in Epidemiology & Public Health (CIBERESP), Carlos III Institute of Health, Avenida Monforte de Lemos 5, 28029 Madrid, Spain

\textsuperscript{c} Cancer Epidemiology Research Group. Oncology and Hematology Area. IIS Puerta de Hierro (IDIPHIM), Manuel de Falla 1, 28222 Madrid, Spain

\textsuperscript{d} Public Health Division of Gipuzkoa, BioDonostia Research Institute, Paseo Dr Beguiristain s/n, 20014 San Sebastian, Spain

\textsuperscript{e} ISGlobal, Centre for Research in Environmental Epidemiology (CREAL), Carrer del Doctor Aiguader 88, 08003 Barcelona, Spain

\textsuperscript{f} IMIM (Hospital del Mar Medical Research Institute), Carrer del Doctor Aiguader 88, 08003 Barcelona, Spain

\textsuperscript{g} Universitat Pompeu Fabra (UPF), Plaça de la Mercè, 10, 08002 Barcelona, Spain

\textsuperscript{h} Instituto de Biomedicina (IBIOMED). Grupo de Investigación en Interacciones Gen-Ambiente y Salud. Universidad de León, Campus Vegazana, s/n, 24071 León, Spain

\textsuperscript{i} Early Detection Section, Public Health Institute of Navarre, IdiSNA, C/ Irunlarrea, 3, 31008 Pamplona, Spain

\textsuperscript{j} Universidad de Cantabria - IDIVAL, Avenida Cardenal Herrera Oria s/n, 39011 Santander, Spain
Instituto Universitario de Oncología IUOPA. University of Oviedo. Facultad de Medicina, Planta 7, Campus de El Cristo B, 33006, Oviedo, Spain

Cancer Prevention and Control Program, Catalan Institute of Oncology (ICO), IDIBELL, Gran Via, 199-203, 08907, L'Hospitalet de Llobregat, Barcelona, Spain

Department of Clinical Sciences, Faculty of Medicine, University of Barcelona, Campus de Bellvitge, Pavelló de Govern, Feixa Llarga s/n, 08907, Barcelona, L'Hospitalet del Llobregat, Spain

Research Group on Statistics, Econometrics and Health (GRECS), Universitat de Girona, Campus Montilivi, 17071 Girona, Spain

Department of Clinical Sciences, Faculty of Medicine, University of Barcelona, Campus de Bellvitge, Pavelló de Govern, Feixa Llarga s/n, 08907, Barcelona, L'Hospitalet del Llobregat, Spain

Research Group on Statistics, Econometrics and Health (GRECS), Universitat de Girona, Campus Montilivi, 17071 Girona, Spain

Epidemiology Unit and Girona Cancer Registry, Oncology Coordination Plan, Department of Health, Autonomous Government of Catalonia, Catalan Institute of Oncology, Girona, Spain

Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunitat Valenciana FISABIO–Salud Pública, Valencia. Avda. Cataluña, 21, 46020, Valencia, Spain

Centre for Research in Health and Environment (CYSMA), Universitat de Huelva, Campus Universitario de El Carmen, 21071, Huelva, Spain

Departments of Epidemiology and Evaluation-IMIM (Hospital del Mar Medical Research Institute), Carrer del Doctor Aiguader 88, 08003, Barcelona, Spain

Research Network on Health Services in Chronic Diseases (REDISSEC), Carrer del Doctor Aiguader 88, 08003, Barcelona, Spain

Universitat Autonoma de Barcelona, Av. Universitat Autònoma, 20, 08290 Cerdanyola del Vallès, Barcelona, Spain

Breast Cancer Screening Program, Osakidetza-Health Basque Region Service, Avenida Navarra, 4, 20013, San Sebastián, Spain

Nutrition and Metabolism Section, International Agency for Research on Cancer, 150 Cours Albert Thomas, 69372 Lyon, France

*Corresponding author: Elena Boldo, Cancer and Environmental Epidemiology Unit, National Epidemiology Center, Carlos III Institute of Health. Avda. de Monforte de Lemos, 5, 28029, Madrid, Spain.
E-mail addresses: eiboldo@isciii.es (E. Boldo), acastello@isciii.es (A. Castelló),
naragones@isciii.es (N. Aragonés), epicss-san@ej-gv.es (P. Amiano), bperez@isciii.es (B.
Pérez-Gómez), gemma.castano@isglobal.org (G. Castaño-Vinyals),
vicente.martin@unileon.es (V. Martín), mguevare@cfnavarra.es (M. Guevara), c-urtiaga@ej-
gv.es (C. Urtiaga), dierssent@unican.es (T. Dierssen-Sotos), gfernanta@gmail.com (G.
Fernández-Tardón), v.moreno@iconcologia.net (V. Moreno), martasolans@gmail.com (M.
Solans), peiro_ros@gva.es (R. Peiro), rocio.capelo@dbasp.uhu.es (R. Capelo),
inges.gomez@unican.es (I. Gómez-Acebo), jesus.castilla.catalan@cfnavarra.es (J. Castilla),
ajmolt@unileon.es (A.J. Molina), XCastells@parcdesalutmar.cat (X. Castells),
JONEMIREN.ALTZIBARAROTZENA@osakidetza.eus (J.M. Altzibar), vicarvajal@isciii.es (V.
Lope), manolis.kogevinas@isglobal.org (M. Kogevinas), romieu@iarc.fr (I. Romieu),
mpollan@isciii.es (M. Pollán).
Highlights

- The role of meat consumption in breast cancer risk is not completely resolved.
- High total intake of meat and high intake of processed/cured meat are associated with breast cancer.
- Well-cooked and stewed red meat increase the risk of breast cancer.
- Some white-meat cooking practices seem to be related to breast cancer risk.
- Breast cancer risk might be reduced by limiting meat consumption.

Abstract

Objective: To analyse the relationship of the risk of breast cancer (BC) to meat intake, preference regarding degree of cooking (‘doneness’) and cooking methods, using data from a population-based case-control study (MCC-Spain).

Study design: 1,006 histologically confirmed incident BC cases and 1,370 controls were recruited in 10 Spanish provinces. Participants were 23-85 years old. They answered an epidemiological survey and a food frequency questionnaire. BC risk was assessed overall, by menopausal status and by pathological subtypes, using logistic and multinomial regression mixed models adjusted for known confounding factors and including province as a random effects term.

Main outcome measures: Breast cancer and pathological subtype.

Results: High total intake of meat (OR_{Q4-Q1} (95% IC) = 1.39 (1.03-1.88)) was associated with increased BC risk among post-menopausal women. Similar results were found for processed/cured meat (OR_{Q4-Q1} (95% IC) = 1.47 (1.10-1.97)), and this association was particularly strong for triple-negative tumours (ER-, PR- and HER2-) (OR_{Q4-Q1} (95% IC) = 2.52 (1.15-5.49)). Intakes of well-done (OR_{well-done vs rare} (95% CI) = 1.62 (1.15-2.30)) and stewed (OR (95% CI) = 1.49 (1.20-1.84)) red meat were associated with increased BC risk, with a high risk observed for HR+ tumours (ER+/PR+ and HER2-). Pan-fried/bread-coated fried white meat, but not doneness preference, was associated with an increased BC risk for all women (OR (95% CI) = 1.49 (1.20-1.84)).
CI) = 1.38 (1.14-1.65)), with a stronger association for pre-menopausal women (OR (95% CI) = 1.78 (1.29-2.46)).

**Conclusion:** The risk of developing BC could be reduced by moderating the consumption of well-done or stewed red meat, pan-fried/bread-coated fried white meat and, especially, processed/cured meat.

**Keywords**
Breast cancer; meat intake; processed meat; cured meat; degree of cooking; cooking methods

**Abbreviations**
BC: breast cancer; BMI: body mass index; FFQ: food frequency questionnaire; MCC-Spain: multicase-control study on common tumours in Spain; OR: odds ratio; 95% CI: 95% confidence interval; SD: standard deviation; IQR: interquartile interval; METs: metabolic equivalents; p-int: p-value for the interaction; p-het: p-value for the heterogeneity; p-trend: p-value for trend; HR+: hormone receptor positive tumours; HER2+: human epidermal growth factor receptor 2 tumours; TN: triple-negative tumours
1. Introduction

Breast cancer (BC) is the most common cancer among women worldwide, and constitutes the leading cause of cancer death among women in medium/high-income countries [1]. In Spain, 6,264 women died from this disease in 2012, and BC accounted for 28% of all cancers in 2015 [2].

The major known risk factors for BC in women include age, family history, and reproductive factors, including early age at menarche, late onset of menopause, nulliparity or first childbirth after age 30 years, overweight and obesity [1]. Even though diet is recognized as a modifiable contributing exposure, no conclusive evidence is available except for body fatness, weight gain and alcohol consumption [3].

The International Agency for Research on Cancer (IARC) classified consumption of red meat as probably carcinogenic to humans (Group 2A), and processed meat as carcinogenic to humans (Group 1) in 2015 [4]. This evaluation was based on consistent associations between consumption of red meat and colorectal, pancreatic and prostate cancer, and processed meat linked to colorectal and stomach cancer. However, the World Cancer Research Fund and the American Institute for Cancer Research (WCRF/AICR) still consider the evidence about its relationship with BC risk as limited [3].

On the other hand, cooked and processed meat can also be a source of several known mutagens, heterocyclic aromatic amines (HCA), and polycyclic aromatic hydrocarbons (PAH), depending on cooking methods, temperatures and duration [5–7]. However, meat cooking practices (methods and degree of cooking, or ‘doneness’) are research gaps in evaluating the relationship of meat and BC, and these factors may partly explain the heterogeneity found among studies.

Therefore, we investigated the role of meat intake, cooking methods and meat doneness in relation to BC, using data from the Spanish multicase-control study (MCC-Spain).
2. Methods

2.1. Study design and population

MCC-Spain [8] is a population-based multicenter case-control study designed to evaluate etiological factors for common cancers in Spain. Between 2008 and 2013, more than 10,000 subjects aged 20-85 years were enrolled in 23 hospitals and primary care centers in 12 Spanish provinces. Participants had to be able to answer the questionnaire and to have resided in the study area in the previous 6 months. The protocol of MCC-Spain was approved by each of the Ethics Committees of the participating institutions. All participants signed an informed consent prior to their inclusion in the study. More detailed information can be found elsewhere [8].

MCC-Spain project recruited 1,738 incident BC cases in 10 Spanish provinces (Barcelona, Madrid, Navarra, Guipúzcoa, León, Asturias, Huelva, Cantabria, Valencia and Girona). Only histologically confirmed incident cases of BC (ICD-10: C50, D05.1, D05.7), with no prior history of the disease, and diagnosed within the recruitment period were included. We also recruited 1,910 healthy women, randomly selected from the listings of primary care centers within the catchment area of the participating hospitals. The response rate was 69% among BC cases and 54% among female controls.

Cases were subclassified according to the local pathology reports [9] in: 1) Hormone receptor positive tumours (HR+): estrogen receptor positive (ER+) and/or progesterone receptor positive (PR+) tumours with luminal human epidermal growth factor receptor 2 negative (HER2-); 2) HER2+ tumours, irrespective of ER or PR results; 3) Triple-negative (TN) tumours with ER-, PR- and HER2-. The ER, PR and HER2 positivity were defined according to American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines [10]. Postmenopausal status was defined as the absence of menstruation in the past 12 months.

2.2. Data collection and exposure assessment

A structured computerized epidemiological questionnaire was administered by trained personnel in a face-to-face interview to collect information on socio-demographic factors, lifestyle, personal/family medical history, reproductive history, and environmental exposures
among other. In order to reduce interviewer bias, experienced professional interviewers –most of them nurses or sociologists- were trained to adhere to the question and answer format strictly, with the same degree of questioning for both cases and controls. The *ad hoc* epidemiological questionnaire was made by the researchers participating in the project after discussing and reaching consensus on the main questions to achieve the MCC-Spain objectives. In many instances, questions were based on questionnaires used in previous studies by the research team. Height and weight at different ages were self-reported, and waist and hip circumference were measured twice with a tape by trained interviewers [8].

At the end of the interview, participants received a semi-quantitative food frequency questionnaire (FFQ) in paper form, to be filled at home (or while they were in the hospital) and returned by mail. This 154-item questionnaire obtained data on the usual food consumption, and was a modified version from a previously validated instrument to include regional products [11]. The FFQ refers to eating habits during the preceding year, and includes meat cooking methods and pictures to establish doneness preference. Cross-check questions on food groups intakes were used to adjust the frequency of foods intake and reduce misreporting of food groups with large numbers of items [12].

Food frequency data were used to derive amount (g/day; g/1000 kcal/day) of each of the individual meat types. Meat products were grouped into the following categories: 1) *white meat*: chicken, turkey, duck and rabbit; 2) *red meat*: beef, veal, pork, lamb, hamburgers (pork or beef), meatballs (pork or beef), liver (beef, pork or chicken) and offal; 3) *processed/cured meat*: meat that has undergone some form of preservation, including sausages, hot dogs, bacon, pate, foie-gras, cooked ham, Spanish cured ham and other Spanish cured sausages (*chorizo*, *fuet*, *salchichón*, *butifarra*, *mortadela*, *botillo*, *cecina*, etc); 4) *total meat*: white, red and processed/cured meat. Methods of cooking meat were grouped into four non-exclusive categories: griddle/barbecued, pan-fried/bread-coated fried, stewed, oven-baking/others. Three levels of doneness preference were considered: rare, medium and well-done.

**2.3. Statistical analyses**
Basic features of the relevant data, such as socio-demographic, lifestyle and meat consumption, were described by summary statistics. Continuous data, normally distributed, were described using mean and standard deviation, and differences were assessed using t-tests. Non-normally distributed continuous variables were described using the median and the interquartile interval (IQR), and differences between cases and controls were tested with non-parametric rank-sum tests. Categorical data were characterized by counts and percentages, and differences between cases and controls were tested using Chi-square tests.

Meat intake was categorized according to the quartile distribution among controls. The association of meat intake in quartiles, meat doneness preference or cooking methods with BC risk was evaluated using logistic mixed regression models, including the province as a random effect term. Adjusted models to derive odds ratios (OR) and 95% confidence intervals (CI) included, as fixed-effects terms: age, educational level, body mass index (BMI) one year before the interview, age at first delivery, age at menarche, previous breast biopsies, family history of BC, menopausal status, smoking status, physical activity, alcohol intake and total energy intake. Meat doneness preference models were further adjusted by the total intake of the corresponding meat group, and non-consumers were excluded. Meat cooking methods (yes/no) were included together in the same model, using the same confounders and also adjusting by total intake of the corresponding meat group. Stratified analyses by menopausal status were conducted including an interaction term in these models, and the significance of the heterogeneity of effects was assessed with the likelihood-ratio test.

Multinomial logistic regression models were adjusted to evaluate the association of meat intake in quartiles, meat doneness preference and cooking methods with BC by pathological subtype. These models took into account the following confounders: age, educational level, body mass index (BMI) one year before the interview, age at first delivery, age at menarche, previous biopsies, family history of BC, menopausal status, smoking status, physical activity, alcohol intake, total energy intake and province. Heterogeneity of effects was tested using a Wald test comparing the coefficients obtained for the different cancer subtypes. To detect multicollinearity in the set of predictor variables, the variance inflation factors (VIFs) were estimated from
regression models for the following continuous variables: age, BMI, physical activity, energy intake, alcohol intake and red meat, white meat and processed/cured meat intake. VIFs indicated non multicollinearity (VIF values between 1.00 and 1.13).

We excluded those participants with missing dietary data (278 cases; 289 controls), extreme reported daily caloric intake (<750 kcal/day or >4,500 kcal/day) (23 cases; 32 controls) or missing information in covariates of interest (118 cases; 219 controls). BC cases that provided dietary information later than 6 months after diagnosis were also excluded (n=313). All statistical analyses were performed using STATA/MP (version 14.1, 2015, StataCorp LP).

3. Results

A total of 1,370 controls and 1,006 cases were analyzed in this study. Compared with the control group, BC cases were younger (56 vs 58 years old), more usually pre-menopausal (37% vs 30%), showed a higher proportion of previous history of breast problems (8% vs 2%), and presented higher daily energy intake than controls (1819 vs 1777 kcal per day) (Table 1). In addition, BC cases reported a slightly higher consumption of processed/cured meat than controls (10.97 vs 9.97 g/day), and specified preference for a higher degree of doneness for red meat (22% vs 19%). Controls had a higher proportion of non-consumers of pan-fried/bread-coated fried white meat (42% vs 34%) or stewed red meat (32% vs 23%) (Table 2).

Only among post-menopausal women we observed an increased risk of BC in women with the highest quartile, compared with the lowest quartile of intake, for total meat (OR_{Q4-Q1} (95% CI) = 1.39 (1.03-1.88); p-int=0.102), and processed/cured meat (OR_{Q4-Q1} (95% CI) = 1.47 (1.10-1.97); p-int= 0.035). Red meat presented a borderline association in post- but not pre-menopausal women (OR_{Q4-Q1} (95% CI) = 1.32 (0.98-1.77); p-int=0.007) (Table 3). The exploration by tumour subtype revealed a positive association of a high consumption of processed/cured meat particularly with TN tumours (OR_{Q4-Q1} (95% CI) = 2.52 (1.15-5.49); p\_trend 0.012), although there was not a significant heterogeneity of effects (p-het=0.517) (Table 4). Sensitivity analysis
including hormonal contraceptives and hormone replacement therapy as confounders yielded very similar results (data not shown).

Regarding red meat doneness preference (Table 5), our results indicated that women who consumed very well done red meat had a 1.62 times higher risk of BC (95% CI = 1.15-2.30; \(p_{\text{trend}} = 0.011\)) than women who consumed it rare done. Such risk appeared to be slightly stronger among post-menopausal women (OR (95% IC) = 1.83 (1.19-2.82)), but the heterogeneity of effects among subgroups was not statistically significant neither for menopausal status nor for BC subtypes. We did not find any significant association with white meat doneness levels and BC risk (results not shown).

In relation to meat cooking methods, pan-fried/bread-coated fried white meat was associated with an increased BC risk for all women (OR (95% CI) = 1.38 (1.14-1.65)), with a stronger association for pre-menopausal women (OR (95% CI) = 1.78 (1.29-2.46); \(p\)-int: 0.059) (Table 6) and no significant differences by BC subtype (Supplementary Table S1). Stewed red meat was associated with an increased BC risk for all women (OR (95% CI) = 1.49 (1.20-1.84)), with no difference by menopausal status (\(p\)-int: 0.476), but mostly limited to HR+ tumours (OR (95% CI) = 1.80 (1.40-2.32); \(p\)-het: 0.007) (Table 6). Sensitivity analyses excluding non-consumers of the corresponding meat group yielded very similar results (data not shown).

4. Discussion

Our results indicate that post-menopausal BC risk was associated with total (>51 g/1000 kcal/day), red (>25 g/1000 kcal/day), and processed/cured (>14 g/1000 kcal/day) meat intake. The detrimental effect of a high consumption of processed/cured meat was particularly strong for TN tumours. Regarding red meat intake, we also observed higher risk of BC with preference for medium/well-doneness while among cooking methods, stewing was specifically linked to higher risk of HR+ tumours. In contrast, total intake of white meat or its doneness preference did not seem to have an effect on BC risk, but the consumption of pan-fried/bread-coated fried
white meat intake was associated with overall BC risk, with a stronger effect among pre-menopausal women.

Although many epidemiologic studies have been conducted to evaluate dietary factors with BC etiology, only a few of them have investigated the relationship with meat intake, including cooking practices, with inconsistent findings. In two large cohort studies, the Black Women’s Health Study (n= 52,062) [13] and the Swedish Mammography Cohort (n= 61,433) [14], no associations were observed between BC risk and total meat [13], total red meat, fresh red meat or processed meat intake [14], regardless of the menopausal and hormone receptor status. Otherwise, recent meta-analyses of prospective studies suggested that higher intake of red and/or processed meat may increase the incidence of BC [15]. Our findings support an association between BC risk and red meat intake, as other studies reported [5,6], and red meat cooked at high temperatures, in line with some previous studies [6,14,16]. In our study, this relationship was stronger among post-menopausal women, similarly to the results published in the Nashville Breast Health Study, a population-based case-control study with more participants and lower meat intake than our study [6]. Moreover, TN BC, associated with a poor prognosis, has been recently associated with animal fat intake and meat consumption [17]. However, the Black Women’s Health Study [13] reported no statistically significant associations of meat intake –including red meat, processed meat, and white meat- with BC by menopausal or hormone receptor status, but dietary patterns and meat-eating habits in African-American women could differ from the participants in this study. Finally, a higher processed red meat intake was associated with higher BC risk in postmenopausal women, in agreement with other studies [18], and in TN tumours. A recent meta-analysis, based on twelve cohort studies, revealed that BC risk increased by 9% per 50 g/day of processed meat [15].

Meat cooking practices may vary across populations, which may partly explain the observed heterogeneity among epidemiological studies. It is difficult to disentangle the influence of various meat cooking methods on BC because participants tend to use different methods. We observed different impact of the meat cooking methods on BC risk by type of meat applying mutual adjustment. First, stewed red meat, heated for a prolonged time, has emerged as a risk
factor for BC, especially for HR+ tumours. This cooking method has been previously associated with nasopharynx [5], stomach [5] and colorectal cancer [19], but not with BC risk. A description on the traditional Spanish stewing and a possible mechanism of carcinogenesis can be found in de Batlle et al.[19]. In summary, carcinogenic compounds produced during the first cooking phase, browned at high temperature, could remain in the sauce during the second phase, cooked for a long time at low temperature.

In our analysis, fried white meat –including buttered, breaded or floured meat- , but not fried red meat, was associated with a higher BC risk, especially in pre-menopausal women. Fried red meat has been associated with a higher risk of cancer of oral cavity, pharynx, esophagus [5] and pre-menopausal BC [20]. Other studies identified fried red meat as a risk factor for pre-menopausal BC [20] or ER+/PR- tumours [14]. High intake of fried meat was also reported to be positively associated with BC in a prospective cohort study in Finland [21], and in several case-control studies [22]. Pan-frying involves cooking meat at high temperature and low moisture conditions. The amount of emissions cooking and fried-food mutagens is related to methods of cooking as well as cooking temperatures and duration [23]. In addition, frying time, types of breading, flouring or battering materials and frying oil influence the oil absorption, and so fat content and caloric consumption [24]. Finally, overheating and reuse of edible fats/oils induces chemical changes such as increase in formation of trans fatty acids and saturated fatty acids, and decrease in cis-unsaturated fatty acids [25].

Red meat has been described as a potential cause of BC by several mechanistic hypotheses: the generation of carcinogenic by-products (HCAs and PAHs) due to cooking meat at high temperatures [7], animal fat [13], heme iron [4] and the animal sugar molecule N-glycolyneuraminic acid [26], which could promote inflammation, oxidative stress, and tumour formation; hormone residues of the exogenous hormones for growth stimulation in beef cattle, which have high affinity for estrogen or androgen receptors [7], and carcinogenic environmental pollutants present in raw or unprocessed meat, such as heavy metals [27]. Potent human carcinogens present in red meat-rich diets, but not white meat [28], are the N-nitroso compounds (NOCs) [7] -N-nitrosamines or N-nitrosoamides- formed in processed meat
products. In addition, processed meat products contain large amount of salt, that are not naturally present in fresh meat and may play a role in the etiology of several cancers [29]. Finally, white meat generally contains less organic contaminants than red meats [27], which could also partly explain the differences observed for global intake of white and red meat in our findings.

Our study has some limitations that should be borne in mind. We obtained information for case subjects on recent usual dietary habits -one year before diagnosis- and assumed the diet did not change, even though women often decrease the amount of red meat they eat during middle age [30]. Therefore, we could not explore whether meat intake and exposure to meat mutagens at a younger age, particularly during adolescence when the breasts are developing, may affect BC risk [30]. On the other hand, subjects completing questionnaires or being interviewed could have had difficulty in remembering past exposures or personal measurements. Because information was collected by interview or self-reported, it was susceptible to recall bias, interviewer bias, or relied on the completeness or accuracy of recorded information, respectively. These biases decrease the internal validity of the investigation, and were carefully addressed in the MCC-Spain study design. Recall bias arises when a differential response between cases and controls occurs, which can lead to a differential exposure misclassification. Patients with cancer might be more conscious of unhealthy dietary habits than healthy participants and changes in dietary habits after diagnosis might also influence their responses to the FFQ. To minimize this bias, some questions about general dietary habits were included in the questionnaire, and used to adjust the responses to the FFQ following the methodology described in Calvert et al. [12]. Additionally, only cases that answered to the questionnaire within the 6 months following the diagnosis were included. We also recognize that self-reported height and weight could be also affected by response or recall bias when estimating BMI the year before BC diagnosis. However, as expected BMI was associated with BC risk only among post-menopausal women, and the consistency and strength of these associations make it unlikely that the recall bias in BC risk estimation could be large in relation to other possible uncertainties. Moreover, interviewer bias occurs when the interviewer asks leading questions or has an inconsistent interview approach between cases and controls. We implemented a
standardized interview with well-trained professional interviewers to reduce this bias. Furthermore, missing values on key variables were completed through subsequent telephone contact. Finally, although all major known risk factors for BC were adjusted for, it is possible that some residual confounding effect may remain.

Strengths of the present study include the recruitment of histologically confirmed incident BC cases, and the use of a detailed FFQ to assess intake of different types of meat, doneness preferences and meat preparation. Most previous studies did not assess meat intake by cooking methods and doneness levels. Moreover, the geographic location of the recruited participants, coming from 10 provinces from the North, South, West and East of the country, ensured the variability in exposure due to different diets coexisting within Spain. Last, we could explore the influence of menopausal status and tumour subtype on the association as the number of participants was sufficiently large to detect differences. This point is especially important because data on meat intake in relation to BC pathological subtypes are really scarce. Although BC is a heterogeneous disease with different etiologies, few studies considered hormone receptor status and HER2 overexpression in their analyses.

5. Conclusion

Our study provides support for the importance of diet in BC prevention, and adds more evidence on the possible role of meat consumption on this tumour. According to our results, associations between meat intake and BC could differ according to type of meat consumed, degree of doneness, and cooking method. The risk of developing BC could be reduced by moderating the consumption of red meat, especially very cooked or stewed, pan-fried/bread-coated fried white meat, as well as processed/cured meat.
Contributors

This research has been conducted by a multi-center group (MCC-Spain).

Elena Boldo participated in the study concept and design, database depuration, analysis and interpretation of the data, and drafting and critical revision of the manuscript.

Adela Castelló participated in the study concept and design, analysis and critical revision of the manuscript.

Nuria Aragonés participated in the study concept and design, acquisition of the data and critical revision of the manuscript.

Pilar Amiano participated in the study concept and design, acquisition of the data, interpretation of the data and critical revision of the manuscript.

Beatriz Pérez-Gómez participated in the study concept and design, acquisition of the data, database depuration, interpretation of the data and critical revision of the manuscript.

Gemma Castaño-Vinyals participated in the study concept and design, acquisition of data and critical revision of the manuscript.

Vicente Martín obtained funding to support the research work submitted, and participated in the study concept and design, acquisition of data and critical revision of the manuscript.

Marcela Guevara participated in the study concept and design and critical revision of the manuscript.

Carmen Urtiaga participated in the study concept and design and critical revision of the manuscript.

Trinidad Dierssen-Sotos participated in the study concept and design, acquisition of data and critical revision of the manuscript.

Guillermo Fernández-Tardón participated in the study concept and design and critical revision of the manuscript.

Victor Moreno participated in the study concept and design, acquisition of data and critical revision of the manuscript.

Marta Solans participated in the study concept and design and critical revision of the manuscript.
Rosanna Peiró participated in the study concept and design, acquisition of data and critical revision of the manuscript.

Rocío Capelo participated in the study concept and design, acquisition of data and critical revision of the manuscript.

Inés Gómez-Acebo participated in the study concept and design, acquisition of data and critical revision of the manuscript.

Jesús Castilla participated in the study concept and design, acquisition of data and critical revision of the manuscript.

Antonio José Molina participated in the study concept and design and critical revision of the manuscript.

Xavier Castells participated in the study concept and design and critical revision of the manuscript.

Jone M. Altzibar participated in the study concept and design and critical revision of the manuscript.

Virginia López-Carvajal participated in the study concept and design and critical revision of the manuscript.

Manolis Kogevinas obtained funding to support the research work submitted, and participated in the study concept and design, acquisition of data and critical revision of the manuscript.

Isabelle Romieu participated in the study concept and design, interpretation of the data, and critical revision of the manuscript.

Marina Pollán obtained funding to support the research work submitted, and participated in the study concept and design, acquisition of data, and drafting and critical revision of the manuscript.

All authors saw and approved the final version of the submitted work.

Conflict of interest

The authors declare that they have no conflict of interest.

This article presents independent research. The views expressed are those of the authors and not necessarily those of the Carlos III Institute of Health.
Funding

This study was supported by Carlos III Institute of Health grants (PI12/00488, PI12/00265, PI12/00715, and PI12/01270, PI09/00773, PI09/1903, PS09/01286, PI08/1770, PI11/02213 (Girona)), the Spanish Ministry of Economy and Competitiveness (IUCI-2014-20900), the Spanish Federation of Breast Cancer Patients (FECMA: EPY 1169-10), the Association of Women with Breast Cancer from Elche (AMACMEC:EPY 1394/15), by Fundación Marqués de Valdecilla (grant API 10/09), by Junta de Castilla y León LE22A10-2, and by Acción Transversal del Cancer, approved by the Spanish Ministry Council on October 11, 2007. Carlos III Institute of Health, co-funded by FEDER funds –a way to build Europe– PI14/00613. Catalan Government DURSI grant 2014SGR647. University of Oviedo, IUOPA and Fundación Caja de Asturias. Elena Boldo was supported by a grant from the Ministry of Economy and Competitiveness (Bolsa de Ampliación de Estudios. Acción Estratégica de Salud del Plan Nacional I+D+i 2008-2011). None of the funders played any role in conducting research or writing the paper.

Ethical approval

The protocol of MCC-Spain was approved by each of the Ethics Committees of the participating institutions. The specific study reported here was approved by the Instituto de Salud Carlos III Ethics Committee (reference CEI PI 44_2012). All participants were informed about the study objectives and gave written informed consent.

Provenance and peer review

This article has undergone peer review.

Research data (data sharing and collaboration)

There are no linked research data sets for this paper. The authors do not have permission to share participants’ personal data.
References


List of tables

Table 1. Socio-demographic and other baseline characteristics for controls and breast cancer cases in MCC-Spain study

Table 2. Meat intake, meat doneness preference and meat cooking methods for controls and breast cancer cases in MCC-Spain study

Table 3. Adjusted odds ratios for the association between breast cancer incidence and quartile of meat intake (g/1000kcal/day), by menopausal status

Table 4. Adjusted odds ratios for the association between breast cancer incidence and quartile of meat intake (g/1000kcal/day), by tumor subtype

Table 5. Adjusted odds ratios for the association between breast cancer incidence and red meat doneness preference among meat consumers, by menopausal status and tumor subtype

Table 6. Adjusted odds ratios for the association between breast cancer incidence and meat cooking methods, by tumor subtype

Supplementary Table S1. Adjusted odds ratios for the association between breast cancer incidence and meat cooking methods, by menopausal status

Table 1. Socio-demographic and other baseline characteristics for controls and breast cancer cases in MCC-Spain study

<table>
<thead>
<tr>
<th></th>
<th>Controls n=1,370</th>
<th>Breast cancer cases n=1,006</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kcal/day), mean (sd)</td>
<td>1777.11 (516.85)</td>
<td>1819.33 (519.61)</td>
<td>0.050</td>
</tr>
<tr>
<td>Alcohol intake (g/day), median (IQR)</td>
<td>1.69 (0.00;7.92)</td>
<td>1.81 (0.00;7.92)</td>
<td>0.143</td>
</tr>
<tr>
<td>BMI (kg/m²), mean (sd)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-menopausal</td>
<td>24.38 (4.84)</td>
<td>23.56 (3.58)</td>
<td>0.008</td>
</tr>
<tr>
<td>Post-menopausal</td>
<td>26.04 (4.70)</td>
<td>26.93 (4.81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical activity (METs), median (IQR)</td>
<td>55.00 (0.00;194.00)</td>
<td>34.80 (0.00;194.90)</td>
<td>0.081</td>
</tr>
<tr>
<td>Age (years), mean (sd)</td>
<td>58.25 (12.55)</td>
<td>56.04 (11.96)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never Smoker</td>
<td>793 (58)</td>
<td>545 (54)</td>
<td>0.059</td>
</tr>
<tr>
<td>Former Smoker</td>
<td>292 (21)</td>
<td>211 (21)</td>
<td></td>
</tr>
<tr>
<td>Current Smoker</td>
<td>285 (21)</td>
<td>250 (25)</td>
<td></td>
</tr>
<tr>
<td>Education, n (%)</td>
<td></td>
<td></td>
<td>0.083</td>
</tr>
<tr>
<td>No formal Education</td>
<td>193 (14)</td>
<td>129 (13)</td>
<td></td>
</tr>
<tr>
<td>Primary School</td>
<td>405 (30)</td>
<td>337 (33)</td>
<td></td>
</tr>
<tr>
<td>Secondary School</td>
<td>449 (33)</td>
<td>337 (33)</td>
<td></td>
</tr>
<tr>
<td>University or more</td>
<td>323 (24)</td>
<td>203 (20)</td>
<td></td>
</tr>
<tr>
<td>Previous breast biopsies, n (%)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
No 1339 (98) 928 (92)
Yes 31 (2) 78 (8)

Family history of BC, n (%) <0.001
None 1171 (85) 749 (74)
2nd Degree 76 (6) 111 (11)
One of 1st degrees 115 (8) 127 (13)
More than one of 1st degree 8 (1) 19 (2)

Age (years) at menarche, mean (sd) 0.573
12.84 (1.58) 12.81 (1.57)

Age (years) at first delivery, n (%) 0.675
25-29 444 (32) 320 (32)
<20 48 (4) 41 (4)
20-24 327 (24) 230 (23)
>29 293 (21) 205 (20)

Nulliparous 258 (19) 210 (21)

Menopausal Status, n (%) 0.001
Pre-menopausal 412 (30) 369 (37)
Post-menopausal 958 (70) 637 (63)

Pathologic BC subtypes, n (%) 0.01
ER+/PR+ and HER2- 685 (75)
HER2+ 160 (17)
ER-,PR- and HER2- 71 (8)

Full et al. 2020. *Figure 1. Medication use, n (%) in mammography and routine.***

Table 2. Meat intake, meat doneness preference and meat cooking methods for controls and breast cancer cases in MCC-Spain study

<table>
<thead>
<tr>
<th></th>
<th>Controls n= 1,370</th>
<th>Breast cancer cases n= 1,006</th>
<th>p-valuea</th>
<th>p-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DAILY INTAKE</strong></td>
<td>n (%) or mean (sd)</td>
<td>n (%) or mean (sd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-consumers</td>
<td>23 (2%)</td>
<td>8 (1%)</td>
<td>0.061</td>
<td>0.095</td>
</tr>
<tr>
<td>Intake (g/day)</td>
<td>40.84 (20.22)</td>
<td>41.83 (19.08)</td>
<td>0.226</td>
<td>0.628</td>
</tr>
<tr>
<td>White meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-consumers</td>
<td>66 (5%)</td>
<td>34 (3%)</td>
<td>0.085</td>
<td>0.185</td>
</tr>
<tr>
<td>Intake (g/day)</td>
<td>12.30 (10.05)</td>
<td>11.87 (8.40)</td>
<td>0.270</td>
<td>0.200</td>
</tr>
<tr>
<td>Red meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-consumers</td>
<td>68 (5%)</td>
<td>33 (3%)</td>
<td>0.044</td>
<td>0.081</td>
</tr>
<tr>
<td>Intake (g/day)</td>
<td>18.57 (13.25)</td>
<td>18.99 (12.13)</td>
<td>0.426</td>
<td>0.826</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Processed/cured meat</th>
<th>n (%) or mean (sd)</th>
<th>n (%) or mean (sd)</th>
<th>p-valuea</th>
<th>p-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-consumers</td>
<td>56 (4%)</td>
<td>26 (3%)</td>
<td>0.047</td>
<td>0.101</td>
</tr>
<tr>
<td>Intake (g/day)</td>
<td>9.97 (7.52)</td>
<td>10.97 (8.33)</td>
<td>0.002</td>
<td>0.017</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DONENESS PREFERENCE</th>
<th>n (%)</th>
<th>n (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>White meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rare</td>
<td>72 (6%)</td>
<td>37 (4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>808 (65%)</td>
<td>599 (66%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well-done</td>
<td>360 (29%)</td>
<td>275 (30%)</td>
<td>0.181</td>
<td>0.347</td>
</tr>
</tbody>
</table>

**Table 2.** Alcohol intake at age 30-40 or current intake if age<30
**Table 1.** BMI one year before recruitment.
**Table 2.** Physical activity during the previous 10 years (excluding 2 years before recruitment).
<table>
<thead>
<tr>
<th></th>
<th>Red meat</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rare</td>
<td>Medium</td>
<td>Well-done</td>
</tr>
<tr>
<td></td>
<td>150 (12%)</td>
<td>852 (69%)</td>
<td>230 (19%)</td>
</tr>
<tr>
<td></td>
<td>80 (9%)</td>
<td>639 (69%)</td>
<td>203 (22%)</td>
</tr>
</tbody>
</table>

**COOKING METHODS**

<table>
<thead>
<tr>
<th></th>
<th>n (%) or mean (sd)</th>
<th>n (%) or mean (sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### White meat

<table>
<thead>
<tr>
<th>Griddle-grilled barbecued</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-consumers</td>
<td>354 (26%)</td>
<td>232 (23%)</td>
</tr>
<tr>
<td>Intake (g/day)</td>
<td>4.16 (5.82)</td>
<td>3.77 (4.92)</td>
</tr>
<tr>
<td>Pan-fried/bread-coated fried</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-consumers</td>
<td>580 (42%)</td>
<td>342 (34%)</td>
</tr>
<tr>
<td>Intake (g/day)</td>
<td>2.00 (3.26)</td>
<td>2.33 (3.23)</td>
</tr>
<tr>
<td>Stewed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-consumers</td>
<td>365 (27%)</td>
<td>233 (23%)</td>
</tr>
<tr>
<td>Intake (g/day)</td>
<td>2.69 (3.52)</td>
<td>2.68 (3.26)</td>
</tr>
<tr>
<td>Oven-baked/other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-consumers</td>
<td>494 (36%)</td>
<td>346 (34%)</td>
</tr>
<tr>
<td>Intake (g/day)</td>
<td>1.90 (3.60)</td>
<td>1.75 (2.42)</td>
</tr>
</tbody>
</table>

### Red meat

<table>
<thead>
<tr>
<th>Griddle-grilled barbecued</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-consumers</td>
<td>212 (15%)</td>
<td>132 (13%)</td>
</tr>
<tr>
<td>Intake (g/day)</td>
<td>6.92 (7.01)</td>
<td>6.67 (6.56)</td>
</tr>
<tr>
<td>Pan-fried/bread-coated fried</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-consumers</td>
<td>540 (39%)</td>
<td>348 (35%)</td>
</tr>
<tr>
<td>Intake (g/day)</td>
<td>3.44 (5.15)</td>
<td>3.80 (5.39)</td>
</tr>
<tr>
<td>Stewed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-consumers</td>
<td>432 (32%)</td>
<td>233 (23%)</td>
</tr>
<tr>
<td>Intake (g/day)</td>
<td>3.01 (4.00)</td>
<td>3.14 (3.71)</td>
</tr>
<tr>
<td>Oven-baked/other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-consumers</td>
<td>692 (51%)</td>
<td>489 (49%)</td>
</tr>
<tr>
<td>Intake (g/day)</td>
<td>1.26 (2.51)</td>
<td>1.24 (2.08)</td>
</tr>
</tbody>
</table>

Differences assessed using Pearson Chi-square test or Student’s t-test as appropriate.

Mean intakes include non-consumers.

Cooking methods are non-exclusive (each participant could report using more than one method).

- \(^a\) p-value without adjusted variables.
- \(^b\) p-value adjusted by age, province and educational level.
Table 3. Adjusted odds ratios for the association between breast cancer incidence and quartile of meat intake (g/1000kcal/day), by menopausal status

<table>
<thead>
<tr>
<th></th>
<th>All women</th>
<th></th>
<th>Pre-menopausal</th>
<th></th>
<th>Post-menopausal</th>
<th></th>
<th>p-int</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n= 2,376</td>
<td>Controls</td>
<td>Cases</td>
<td>OR (95%CI)</td>
<td>Controls</td>
<td>Cases</td>
<td>OR (95%CI)</td>
</tr>
<tr>
<td>Total meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 &lt;27.85</td>
<td></td>
<td>345</td>
<td>227</td>
<td>1</td>
<td>64</td>
<td>66</td>
<td>1</td>
</tr>
<tr>
<td>Q2 27.85-38.35</td>
<td></td>
<td>339</td>
<td>225</td>
<td>0.99 (0.78;1.27)</td>
<td>95</td>
<td>86</td>
<td>0.94 (0.59;1.50)</td>
</tr>
<tr>
<td>Q3 38.35-51.10</td>
<td></td>
<td>344</td>
<td>284</td>
<td>1.21 (0.95;1.54)</td>
<td>113</td>
<td>108</td>
<td>0.98 (0.63;1.54)</td>
</tr>
<tr>
<td>Q4 &gt;=51.10</td>
<td></td>
<td>342</td>
<td>270</td>
<td>1.15 (0.90;1.48)</td>
<td>140</td>
<td>109</td>
<td>0.79 (0.50;1.23)</td>
</tr>
<tr>
<td>P-trend</td>
<td></td>
<td></td>
<td></td>
<td>0.120</td>
<td></td>
<td></td>
<td>0.289</td>
</tr>
<tr>
<td>White meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 &lt;6.41</td>
<td></td>
<td>349</td>
<td>204</td>
<td>1</td>
<td>93</td>
<td>66</td>
<td>1</td>
</tr>
<tr>
<td>Q2 6.41-10.0</td>
<td></td>
<td>345</td>
<td>308</td>
<td>1.58 (1.24;2.00)</td>
<td>97</td>
<td>121</td>
<td>1.95 (1.28;3.00)</td>
</tr>
<tr>
<td>Q3 10.03-15.54</td>
<td></td>
<td>338</td>
<td>264</td>
<td>1.38 (1.08;1.78)</td>
<td>97</td>
<td>96</td>
<td>1.52 (0.98;2.37)</td>
</tr>
<tr>
<td>Q4 &gt;=15.54</td>
<td></td>
<td>338</td>
<td>230</td>
<td>1.18 (0.91;1.53)</td>
<td>125</td>
<td>86</td>
<td>1.07 (0.69;1.65)</td>
</tr>
<tr>
<td>P-trend</td>
<td></td>
<td></td>
<td></td>
<td>0.445</td>
<td></td>
<td></td>
<td>0.618</td>
</tr>
<tr>
<td>Red meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 &lt;9.66</td>
<td></td>
<td>347</td>
<td>222</td>
<td>1</td>
<td>82</td>
<td>62</td>
<td>1</td>
</tr>
<tr>
<td>Q2 9.66-16.29</td>
<td></td>
<td>339</td>
<td>261</td>
<td>1.14 (0.90;1.46)</td>
<td>90</td>
<td>96</td>
<td>1.38 (0.88;2.18)</td>
</tr>
<tr>
<td>Q3 16.29-25.31</td>
<td></td>
<td>340</td>
<td>256</td>
<td>1.10 (0.86;1.41)</td>
<td>102</td>
<td>113</td>
<td>1.53 (0.99;2.38)</td>
</tr>
<tr>
<td>Q4 &gt;=25.31</td>
<td></td>
<td>344</td>
<td>267</td>
<td>1.15 (0.90;1.47)</td>
<td>138</td>
<td>98</td>
<td>0.96 (0.62;1.49)</td>
</tr>
<tr>
<td>P-trend</td>
<td></td>
<td></td>
<td></td>
<td>0.347</td>
<td></td>
<td></td>
<td>0.678</td>
</tr>
<tr>
<td>Processed/cured meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 &lt;4.86</td>
<td></td>
<td>347</td>
<td>225</td>
<td>1</td>
<td>73</td>
<td>61</td>
<td>1</td>
</tr>
<tr>
<td>Q2 4.86-8.83</td>
<td></td>
<td>334</td>
<td>266</td>
<td>1.16 (0.91;1.48)</td>
<td>83</td>
<td>103</td>
<td>1.48 (0.93;2.34)</td>
</tr>
<tr>
<td>Q3 8.83-13.65</td>
<td></td>
<td>351</td>
<td>213</td>
<td>0.87 (0.68;1.12)</td>
<td>116</td>
<td>83</td>
<td>0.84 (0.53;1.33)</td>
</tr>
<tr>
<td>Q4 &gt;=13.65</td>
<td></td>
<td>338</td>
<td>302</td>
<td>1.27 (1.00;1.62)</td>
<td>140</td>
<td>122</td>
<td>1.02 (0.66;1.57)</td>
</tr>
<tr>
<td>P-trend</td>
<td></td>
<td></td>
<td></td>
<td>0.221</td>
<td></td>
<td></td>
<td>0.375</td>
</tr>
</tbody>
</table>

Abbreviations: OR= odds ratios; CI= confidence interval; p-int= P value of the interaction term between menopausal status and the corresponding variable. Adjusted for age, province, educational level, BMI one year before the interview, age at first delivery, age at menarche, previous breast biopsies, family history of BC, menopausal status, smoking, physical activity, alcohol intake and total energy intake.
Table 4. Adjusted odds ratios for the association between breast cancer incidence and quartile of meat intake (g/1000kcal/day), by tumor subtype

<table>
<thead>
<tr>
<th></th>
<th>Controls n= 1,370</th>
<th>HR+ n= 685</th>
<th>HER2+ n= 160</th>
<th>TN n= 71</th>
<th>p-het</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total meat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 &lt;27.85</td>
<td>345</td>
<td>156</td>
<td>1</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td>Q2 27.85-38.35</td>
<td>339</td>
<td>145</td>
<td>0.93 (0.70;1.24)</td>
<td>45</td>
<td>1.41 (0.86;2.31)</td>
</tr>
<tr>
<td>Q3 38.35-51.10</td>
<td>344</td>
<td>191</td>
<td>1.16 (0.88;1.53)</td>
<td>52</td>
<td>1.56 (0.96;2.52)</td>
</tr>
<tr>
<td>Q4 &gt;=51.10</td>
<td>342</td>
<td>193</td>
<td>1.19 (0.89;1.58)</td>
<td>32</td>
<td>0.95 (0.55;1.64)</td>
</tr>
<tr>
<td>P-trend</td>
<td>0.101</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>White meat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 &lt;6.41</td>
<td>349</td>
<td>142</td>
<td>1</td>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td>Q2 6.41-10.03</td>
<td>345</td>
<td>208</td>
<td>1.52 (1.16;2.00)</td>
<td>50</td>
<td>1.87 (1.14;3.06)</td>
</tr>
<tr>
<td>Q3 10.03-15.54</td>
<td>338</td>
<td>174</td>
<td>1.27 (0.95;1.69)</td>
<td>45</td>
<td>1.75 (1.04;2.93)</td>
</tr>
<tr>
<td>Q4 &gt;=15.54</td>
<td>338</td>
<td>161</td>
<td>1.14 (0.85;1.54)</td>
<td>37</td>
<td>1.44 (0.84;2.48)</td>
</tr>
<tr>
<td>P-trend</td>
<td>0.718</td>
<td></td>
<td></td>
<td></td>
<td>0.833</td>
</tr>
<tr>
<td><strong>Red meat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 &lt;9.66</td>
<td>347</td>
<td>151</td>
<td>1</td>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td>Q2 9.66-16.29</td>
<td>339</td>
<td>182</td>
<td>1.15 (0.87;1.51)</td>
<td>37</td>
<td>0.98 (0.60;1.60)</td>
</tr>
<tr>
<td>Q3 16.29-25.31</td>
<td>340</td>
<td>161</td>
<td>1.01 (0.76;1.34)</td>
<td>51</td>
<td>1.32 (0.83;2.11)</td>
</tr>
<tr>
<td>Q4 &gt;=25.31</td>
<td>344</td>
<td>191</td>
<td>1.20 (0.91;1.59)</td>
<td>36</td>
<td>0.91 (0.55;1.51)</td>
</tr>
<tr>
<td>P-trend</td>
<td>0.343</td>
<td></td>
<td></td>
<td></td>
<td>0.762</td>
</tr>
<tr>
<td><strong>Processed/cured meat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 &lt;4.86</td>
<td>347</td>
<td>151</td>
<td>1</td>
<td>38</td>
<td>1</td>
</tr>
<tr>
<td>Q2 4.86-8.83</td>
<td>334</td>
<td>187</td>
<td>1.21 (0.92;1.59)</td>
<td>43</td>
<td>1.24 (0.77;1.99)</td>
</tr>
<tr>
<td>Q3 8.83-13.65</td>
<td>351</td>
<td>140</td>
<td>0.84 (0.63;1.12)</td>
<td>36</td>
<td>0.90 (0.55;1.48)</td>
</tr>
<tr>
<td>Q4 &gt;=13.65</td>
<td>338</td>
<td>207</td>
<td>1.27 (0.97;1.68)</td>
<td>43</td>
<td>1.09 (0.67;1.76)</td>
</tr>
<tr>
<td>P-trend</td>
<td>0.355</td>
<td></td>
<td></td>
<td></td>
<td>0.012</td>
</tr>
</tbody>
</table>

Abbreviations: HR+= hormone receptor positive tumours; HER2+= human epidermal growth factor receptor 2 tumours; TN= triple-negative tumours; OR= odds ratios; CI= confidence interval; p-het= P value of heterogeneity of effect between pathologic subtypes. Adjusted for age, province, educational level, BMI one year before the interview, age at first delivery, age at menarche, previous breast biopsies, family history of BC, menopausal status, smoking, physical activity, alcohol intake and total energy intake.
Table 5. Adjusted odds ratios for the association between breast cancer incidence and red meat doneness preference among meat consumers, by menopausal status and tumor subtype

<table>
<thead>
<tr>
<th></th>
<th>All women n= 2,154</th>
<th>Pre-menopausal n= 729</th>
<th>Post-menopausal n= 1,425</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls Cases OR (95%CI)</td>
<td>Controls Cases OR (95%CI)</td>
<td>Controls Cases OR (95%CI)</td>
</tr>
<tr>
<td>Rare</td>
<td>150 80 1</td>
<td>44 33 1</td>
<td>106 47 1</td>
</tr>
<tr>
<td>Medium</td>
<td>852 639 1.43 (1.06;1.94)</td>
<td>263 242 1.28 (0.78;2.12)</td>
<td>589 397 1.52 (1.04;2.23)</td>
</tr>
<tr>
<td>Well-done</td>
<td>230 203 1.62 (1.15;2.30)</td>
<td>75 72 1.31 (0.73;2.33)</td>
<td>155 131 1.83 (1.19;2.82)</td>
</tr>
<tr>
<td>p-trend</td>
<td>0.011</td>
<td>0.458</td>
<td>0.009</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Controls Cases OR (95%CI)</th>
<th>HR+ n= 624 Cases OR (95%CI)</th>
<th>HER2+ n= 151 Cases OR (95%CI)</th>
<th>TN n= 64 Cases OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rare</td>
<td>150 58 1</td>
<td>14 1</td>
<td>3 1</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>852 426 1.37 (0.97;1.94)</td>
<td>101 1.26 (0.68;2.31)</td>
<td>48 2.41 (0.72;8.06)</td>
<td></td>
</tr>
<tr>
<td>Well-done</td>
<td>230 140 1.68 (1.13;2.50)</td>
<td>36 1.62 (0.82;3.20)</td>
<td>13 2.19 (0.59;8.14)</td>
<td></td>
</tr>
<tr>
<td>p-trend</td>
<td>0.011</td>
<td>0.132</td>
<td>0.457</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: OR= odds ratios; CI= confidence interval; p-int= P value of the interaction term between menopausal status and the corresponding variable; HR+= hormone receptor positive tumours; HER2+= human epidermal growth factor receptor 2 tumours; TN= triple-negative tumours; OR= odds ratios; p-het= P value of heterogeneity of effect between pathologic subtypes.

Adjusted for age, province, educational level, BMI one year before the interview, age at first delivery, age at menarche, previous breast biopsies, family history of BC, menopausal status, smoking, physical activity, alcohol intake, total energy intake and red meat intake.

Non-consumers were excluded from the analyses.
Table 6. Adjusted odds ratios for the association between breast cancer incidence and meat cooking methods, by tumor subtype

<table>
<thead>
<tr>
<th></th>
<th>Controls n= 1,370</th>
<th>HR+ n= 685</th>
<th>HER2+ n= 160</th>
<th>TN n= 71</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percentage OR (95%CI)</td>
<td>P</td>
<td>Percentage OR (95%CI)</td>
<td>P</td>
</tr>
<tr>
<td><strong>Total meat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Griddle/barbecued</td>
<td>90 92 1.02 (0.69;1.50)</td>
<td>0.926</td>
<td>91 1.14 (0.60;2.14)</td>
<td>0.696</td>
</tr>
<tr>
<td>Pan-fried/bread-coated fried</td>
<td>75 81 1.13 (0.87;1.46)</td>
<td>0.360</td>
<td>79 1.08 (0.69;1.69)</td>
<td>0.732</td>
</tr>
<tr>
<td>Stewed</td>
<td>85 92 1.82 (1.28;2.59)</td>
<td>0.001</td>
<td>87 1.28 (0.73;2.23)</td>
<td>0.389</td>
</tr>
<tr>
<td>Oven-baked/other</td>
<td>75 78 0.97 (0.76;1.25)</td>
<td>0.835</td>
<td>73 0.86 (0.56;1.31)</td>
<td>0.490</td>
</tr>
<tr>
<td><strong>White meat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Griddle/barbecued</td>
<td>74 77 1.06 (0.82;1.36)</td>
<td>0.659</td>
<td>76 1.24 (0.80;1.91)</td>
<td>0.343</td>
</tr>
<tr>
<td>Pan-fried/bread-coated fried</td>
<td>58 66 1.40 (1.13;1.73)</td>
<td>0.002</td>
<td>65 1.32 (0.91;1.91)</td>
<td>0.142</td>
</tr>
<tr>
<td>Stewed</td>
<td>73 77 1.07 (0.84;1.36)</td>
<td>0.570</td>
<td>76 1.19 (0.78;1.80)</td>
<td>0.424</td>
</tr>
<tr>
<td>Oven-baked/other</td>
<td>64 67 0.97 (0.77;1.21)</td>
<td>0.769</td>
<td>59 0.71 (0.49;1.04)</td>
<td>0.078</td>
</tr>
<tr>
<td><strong>Red meat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Griddle/barbecued</td>
<td>85 87 0.95 (0.69;1.29)</td>
<td>0.727</td>
<td>85 1.14 (0.68;1.93)</td>
<td>0.611</td>
</tr>
<tr>
<td>Pan-fried/bread-coated fried</td>
<td>61 66 1.07 (0.86;1.34)</td>
<td>0.553</td>
<td>61 0.84 (0.58;1.23)</td>
<td>0.379</td>
</tr>
<tr>
<td>Stewed</td>
<td>68 79 1.80 (1.40;2.32)</td>
<td>&lt;0.001</td>
<td>69 1.07 (0.72;1.61)</td>
<td>0.732</td>
</tr>
<tr>
<td>Oven-baked/other</td>
<td>49 52 0.93 (0.75;1.15)</td>
<td>0.491</td>
<td>49 0.96 (0.67;1.38)</td>
<td>0.819</td>
</tr>
</tbody>
</table>

Abbreviations: HR+= hormone receptor positive tumours; HER2+= human epidermal growth factor receptor 2 tumours; TN= triple-negative tumours; OR= odds ratios; CI= confidence interval; p-het= P value of heterogeneity of effect between pathologic subtypes.

Adjusted for age, province, educational level, BMI one year before the interview, age at first delivery, age at menarche, previous biopsies, family history of BC, menopausal status, smoking, physical activity, alcohol intake, total energy intake, the corresponding meat group and other meat cooking methods.

Reference category: no intake of the corresponding meat cooking method.