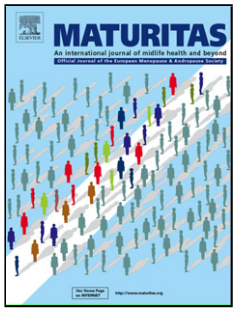

Title: Meat intake, methods and degrees of cooking and breast cancer risk in the MCC-Spain study

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

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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 ($OR_{Q4\text{-}Q1}$ (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 ($OR_{Q4\text{-}Q1}$ (95% IC) = 1.47 (1.10-1.97)), and this association was particularly strong for triple-negative tumours (ER-, PR- and HER2-) ($OR_{Q4\text{-}Q1}$ (95% IC) = 2.52 (1.15-5.49)). Intakes of well-done ($OR_{well\text{-}done\ vs\ rare}$ (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) = 1.38 (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 ($OR_{Q4\text{-}Q1}$ (95% CI) = 1.39 (1.03-1.88); p-int=0.102), and processed/cured meat ($OR_{Q4\text{-}Q1}$ (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 ($OR_{Q4\text{-}Q1}$ (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 ($OR_{Q4\text{-}Q1}$ (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_{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.

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Conflict of interest

The authors declare that they have no conflict of interest.

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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.

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

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List of tables

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

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

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)	12.84 (1.58)	12.81 (1.57)	0.573
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)	
Nuliparous	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 (%)			
ER+/PR+ and HER2-		685 (75)	
HER2+		160 (17)	
ER-,PR- and HER2-		71 (8)	

[a] Alcohol intake at age 30-40 or current intake if age<30

[b] BMI one year before recruitment.

[c] Physical activity during the previous 10 years (excluding 2 years before recruitment).

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

	Controls n= 1,370	**Breast cancer cases n= 1,006**	**p-value[a]**	**p-value[b]**
DAILY INTAKE	**n (%) or mean (sd)**	**n (%) or mean (sd)**		
Total meat				
Non-consumers	23 (2%)	8 (1%)	0.061	0.095
Intake (g/day)	40.84 (20.22)	41.83 (19.08)	0.226	0.628
White meat				
Non-consumers	66 (5%)	34 (3%)	0.085	0.185
Intake (g/day)	12.30 (10.05)	11.87 (8.40)	0.270	0.200
Red meat				
Non-consumers	68 (5%)	33 (3%)	0.044	0.081
Intake (g/day)	18.57 (13.25)	18.99 (12.13)	0.426	0.826
Processed/cured meat				
Non-consumers	56 (4%)	26 (3%)	0.047	0.101
Intake (g/day)	9.97 (7.52)	10.97 (8.33)	0.002	0.017
DONENESS PREFERENCE	**n (%)**	**n (%)**		
White meat				
Rare	72 (6%)	37 (4%)		
Medium	808 (65%)	599 (66%)		
Well-done	360 (29%)	275 (30%)	0.181	0.347

Red meat				
Rare	150 (12%)	80 (9%)		
Medium	852 (69%)	639 (69%)		
Well-done	230 (19%)	203 (22%)	0.011	0.015
COOKING METHODS	**n (%) or mean (sd)**	**n (%) or mean (sd)**		
White meat				
Griddle-grilled barbecued				
Non-consumers	354 (26%)	232 (23%)	0.121	0.367
Intake (g/day)	4.16 (5.82)	3.77 (4.92)	0.085	0.073
Pan-fried/bread-coated fried				
Non-consumers	580 (42%)	342 (34%)	<0.001	0.001
Intake (g/day)	2.00 (3.26)	2.33 (3.23)	0.015	0.039
Stewed				
Non-consumers	365 (27%)	233 (23%)	0.053	0.096
Intake (g/day)	2.69 (3.52)	2.68 (3.26)	0.910	0.987
Oven-baked/other				
Non-consumers	494 (36%)	346 (34%)	0.402	0.914
Intake (g/day)	1.90 (3.60)	1.75 (2.42)	0.225	0.137
Red meat				
Griddle-grilled barbecued				
Non-consumers	212 (15%)	132 (13%)	0.107	0.206
Intake (g/day)	6.92 (7.01)	6.67 (6.56)	0.365	0.281
Pan-fried/bread-coated fried				
Non-consumers	540 (39%)	348 (35%)	0.016	0.051
Intake (g/day)	3.44 (5.15)	3.80 (5.39)	0.101	0.164
Stewed				
Non-consumers	432 (32%)	233 (23%)	<0.001	<0.001
Intake (g/day)	3.01 (4.00)	3.14 (3.71)	0.422	0.604
Oven-baked/other				
Non-consumers	692 (51%)	489 (49%)	0.359	0.476
Intake (g/day)	1.26 (2.51)	1.24 (2.08)	0.832	0.689

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

	All women n= 2,376			Pre-menopausal n= 781			Post-menopausal n= 1,595			
	Controls	Cases	OR (95%CI)	Controls	Cases	OR (95%CI)	Controls	Cases	OR (95%CI)	p-int
Total meat										
Q1 <27.85	345	227	1	64	66	1	281	161	1	0.102
Q2 27.85-38.35	339	225	0.99 (0.78;1.27)	95	86	0.94 (0.59;1.50)	244	139	0.98 (0.73;1.32)	
Q3 38.35-51.10	344	284	1.21 (0.95;1.54)	113	108	0.98 (0.63;1.54)	231	176	1.30 (0.98;1.74)	
Q4 >=51.10	342	270	1.15 (0.90;1.48)	140	109	0.79 (0.50;1.23)	202	161	1.39 (1.03;1.88)	
P-trend			0.120			0.289			0.009	
White meat										
Q1 <6.41	349	204	1	93	66	1	256	138	1	0.239
Q2 6.41-10.0	345	308	1.58 (1.24;2.00)	97	121	1.95 (1.28;3.00)	248	187	1.42 (1.06;1.90)	
Q3 10.03-15.54	338	264	1.38 (1.08;1.78)	97	96	1.52 (0.98;2.37)	241	168	1.33 (0.98;1.79)	
Q4 >=15.54	338	230	1.18 (0.91;1.53)	125	86	1.07 (0.69;1.65)	213	144	1.27 (0.93;1.74)	
P-trend			0.445			0.618			0.192	
Red meat										
Q1 <9.66	347	222	1	82	62	1	265	160	1	0.007
Q2 9.66-16.29	339	261	1.14 (0.90;1.46)	90	96	1.38 (0.88;2.18)	249	165	1.05 (0.79;1.40)	
Q3 16.29-25.31	340	256	1.10 (0.86;1.41)	102	113	1.53 (0.99;2.38)	238	143	0.92 (0.69;1.24)	
Q4 >=25.31	344	267	1.15 (0.90;1.47)	138	98	0.96 (0.62;1.49)	206	169	1.32 (0.98;1.77)	
P-trend			0.347			0.678			0.149	
Processed/cured meat										
Q1 <4.86	347	225	1	73	61	1	274	164	1	0.035
Q2 4.86-8.83	334	266	1.16 (0.91;1.48)	83	103	1.48 (0.93;2.34)	251	163	1.03 (0.78;1.38)	
Q3 8.83-13.65	351	213	0.87 (0.68;1.12)	116	83	0.84 (0.53;1.33)	235	130	0.88 (0.65;1.19)	
Q4 >=13.65	338	302	1.27 (1.00;1.62)	140	122	1.02 (0.66;1.57)	198	180	1.47 (1.10;1.97)	
P-trend			0.221			0.375			0.035	

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

	Controls n= 1,370	HR+ n= 685		HER2+ n= 160		TN n= 71		
		Cases	OR (95%CI)	Cases	OR (95%CI)	Cases	OR (95%CI)	p-het
Total meat								
Q1 <27.85	345	156	1	31	1	18	1	0.473
Q2 27.85-38.35	339	145	0.93 (0.70;1.24)	45	1.41 (0.86;2.31)	14	0.72 (0.35;1.51)	
Q3 38.35-51.10	344	191	1.16 (0.88;1.53)	52	1.56 (0.96;2.52)	18	0.89 (0.44;1.79)	
Q4 >=51.10	342	193	1.19 (0.89;1.58)	32	0.95 (0.55;1.64)	21	1.00 (0.50;2.02)	
P-trend			0.101		0.983		0.833	
White meat								
Q1 <6.41	349	142	1	28	1	18	1	0.354
Q2 6.41-10.03	345	208	1.52 (1.16;2.00)	50	1.87 (1.14;3.06)	23	1.14 (0.59;2.19)	
Q3 10.03-15.54	338	174	1.27 (0.95;1.69)	45	1.75 (1.04;2.93)	18	0.86 (0.42;1.74)	
Q4 >=15.54	338	161	1.14 (0.85;1.54)	37	1.44 (0.84;2.48)	12	0.60 (0.27;1.32)	
P-trend			0.718		0.279		0.156	
Red meat								
Q1 <9.66	347	151	1	36	1	16	1	0.524
Q2 9.66-16.29	339	182	1.15 (0.87;1.51)	37	0.98 (0.60;1.60)	15	0.82 (0.39;1.72)	
Q3 16.29-25.31	340	161	1.01 (0.76;1.34)	51	1.32 (0.83;2.11)	21	1.14 (0.57;2.29)	
Q4 >=25.31	344	191	1.20 (0.91;1.59)	36	0.91 (0.55;1.51)	19	1.05 (0.51;2.15)	
P-trend			0.343		0.944		0.672	
Processed/cured meat								
Q1 <4.86	347	151	1	38	1	10	1	0.517
Q2 4.86-8.83	334	187	1.21 (0.92;1.59)	43	1.24 (0.77;1.99)	15	1.39 (0.61;3.18)	
Q3 8.83-13.65	351	140	0.84 (0.63;1.12)	36	0.90 (0.55;1.48)	20	1.93 (0.87;4.28)	
Q4 >=13.65	338	207	1.27 (0.97;1.68)	43	1.09 (0.67;1.76)	26	2.52 (1.15;5.49)	
P-trend			0.355		0.936		0.012	

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

	All women n= 2,154			Pre-menopausal n= 729			Post-menopausal n= 1,425			
	Controls	Cases	OR (95%CI)	Controls	Cases	OR (95%CI)	Controls	Cases	OR (95%CI)	p-int
Rare	150	80	1	44	33	1	106	47	1	0.634
Medium	852	639	1.43 (1.06;1.94)	263	242	1.28 (0.78;2.12)	589	397	1.52 (1.04;2.23)	
Well-done	230	203	1.62 (1.15;2.30)	75	72	1.31 (0.73;2.33)	155	131	1.83 (1.19;2.82)	
p-trend			0.011			0.458			0.009	

	Controls n= 1,232	HR+ n= 624		HER2+ n= 151		TN n= 64		
		Cases	OR (95%CI)	Cases	OR (95%CI)	Cases	OR (95%CI)	p-het
Rare	150	58	1	14	1	3	1	0.988
Medium	852	426	1.37 (0.97;1.94)	101	1.26 (0.68;2.31)	48	2.41 (0.72;8.06)	
Well-done	230	140	1.68 (1.13;2.50)	36	1.62 (0.82;3.20)	13	2.19 (0.59;8.14)	
p-trend			0.011		0.132		0.457	

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

	Controls n= 1,370		HR+ n= 685		HER2+ n= 160			TN n= 71			
	Percentage		OR (95%CI)	P	Percentage	OR (95%CI)	P	Percentage	OR (95%CI)	P	p-het
Total meat											
Griddle/barbecued	90	92	1.02 (0.69;1.50)	0.926	91	1.14 (0.60;2.14)	0.696	90	1.07 (0.44;2.65)	0.876	0.896
Pan-fried/bread-coated fried	75	81	1.13 (0.87;1.46)	0.360	79	1.08 (0.69;1.69)	0.732	83	1.61 (0.80;3.23)	0.182	0.776
Stewed	85	92	1.82 (1.28;2.59)	0.001	87	1.28 (0.73;2.23)	0.389	86	0.85 (0.39;1.84)	0.685	0.099
Oven-baked/other	75	78	0.97 (0.76;1.25)	0.835	73	0.86 (0.56;1.31)	0.490	69	0.67 (0.37;1.21)	0.181	0.293
White meat											
Griddle/barbecued	74	77	1.06 (0.82;1.36)	0.659	76	1.24 (0.80;1.91)	0.343	77	1.49 (0.77;2.88)	0.238	0.780
Pan-fried/bread-coated fried	58	66	1.40 (1.13;1.73)	0.002	65	1.32 (0.91;1.91)	0.142	63	1.24 (0.72;2.15)	0.435	0.782
Stewed	73	77	1.07 (0.84;1.36)	0.570	76	1.19 (0.78;1.80)	0.424	72	0.83 (0.46;1.49)	0.531	0.567
Oven-baked/other	64	67	0.97 (0.77;1.21)	0.769	59	0.71 (0.49;1.04)	0.078	59	0.73 (0.42;1.26)	0.257	0.266
Red meat											
Griddle/barbecued	85	87	0.95 (0.69;1.29)	0.727	85	1.14 (0.68;1.93)	0.611	86	1.13 (0.52;2.45)	0.756	0.997
Pan-fried/bread-coated fried	61	66	1.07 (0.86;1.34)	0.553	61	0.84 (0.58;1.23)	0.379	69	1.34 (0.75;2.37)	0.320	0.237
Stewed	68	79	1.80 (1.40;2.32)	<0.001	69	1.07 (0.72;1.61)	0.732	69	0.82 (0.46;1.48)	0.517	0.007
Oven-baked/other	49	52	0.93 (0.75;1.15)	0.491	49	0.96 (0.67;1.38)	0.819	52	1.04 (0.61;1.79)	0.877	0.897

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.