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ORIGINAL CONTRIBUTION

Meat intake, cooking methods and doneness and risk of colorectal tumours in the Spanish multicase-control study (MCC-Spain)

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Abstract

Purpose Although there is convincing evidence that red and processed meat intake increases the risk of colorectal cancer (CRC), the potential role of meat cooking practices has not been established yet and could partly explain the current heterogeneity of results among studies. Therefore, we aimed to investigate the association between meat consumption and cooking practices and the risk of CRC in a population-based case–control study.

Methods A total of 1671 CRC cases and 3095 controls recruited in Spain between September 2008 and December 2013 completing a food frequency questionnaire with a meat-specific module were included in the analyses. Odds ratios (OR) and confidence intervals (CI) were estimated by logistic regression models adjusted for known confounders.

Results Total meat intake was associated with increased risk of CRC (OR_{T3-T1} 1.41; 95% CI 1.19–1.67; p_{trend} < 0.001), and similar associations were found for white, red and processed/cured/organ meat. Rare-cooked meat preference was associated with low risk of CRC in red meat ($OR_{rare\ vs.\ medium}$ 0.66; 95% CI 0.51–0.85) and total meat ($OR_{rare\ vs.\ medium}$ 0.56; 95% CI 0.37–0.86) consumers,

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these associations being stronger in women than in men. Griddle-grilled/barbecued meat was associated with an increased CRC risk (total meat: OR 1.45; 95% CI 1.13–1.87). Stewing (OR 1.25; 95% CI 1.04–1.51) and oven-baking (OR 1.18; 95% CI 1.00–1.40) were associated with increased CRC risk of white, but not red, meat.
Conclusions Our study supports an association of white, red, processed/cured/organ and total meat intake with an increased risk of CRC. Moreover, our study showed that cooking practices can modulate such risk.

Keywords Colorectal cancer · Meat · Cooking · Epidemiology

Introduction

It has been estimated that in 2012 colorectal cancer (CRC) was the third most common cancer worldwide in men (746,000 cases, 10.0% of the total) and the second in women (614,000 cases, 9.2%), accounting for 694,000 deaths [1]. In Spain, CRC is estimated to be the third most incident cancer [2] and the second with the highest mortality (15,575 deaths in 2013) [3]. The 2011 World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) Continuous Update Project Report Summary for Colorectal Cancer stated that there is convincing evidence that red meat, processed meat and alcoholic drinks increase the risk of CRC [4]. Subsequent reviews and meta-analyses did not agree on the role of red and processed meat in CRC [5–9], and the recent announcement of the conclusions of the 114th Monograph of the International Agency for Research on Cancer (IARC) [10], dealing with the topic of carcinogenicity of red and processed meat, has captured the attention of stakeholders all around the world.

There are several plausible mechanisms involving meat components that could explain the association between meat intake and CRC risk [11]. Haem iron and nitrites/nitrates from red meat and processed meats can increase the exposure to carcinogenic N-nitroso compounds (NOCs) [11–13]. Additionally, heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) formed during high-temperature meat cooking are also mutagens and carcinogens [11, 14]. Therefore, meat cooking practices could be relevant when assessing the association between meat intake and CRC, and not considering them may partly explain the current heterogeneity of results among studies.

Few studies up to date, have considered both cooking methods and meat doneness in relation to CRC [15–23]. Moreover, findings have not been consistent across studies, probably due to differences in both cooking habits and total amount of meat consumed in the studied populations. Aiming to elucidate the role of meat in CRC, the association between meat and CRC was investigated considering meat cooking methods in the Spanish multicase-control study (MCC-Spain). The MCC-Spain study provides an excellent framework for this purpose, as Spain has a high while still heterogeneous intake of meat, and the multicentric nature of the study allows for a wider range of cooking practices.

Materials and methods

Study design and population

MCC-Spain is a multicentre case–control study with population controls aiming to evaluate the influence of environmental exposures and their interaction with genetic factors in common tumours in Spain (prostate, breast, colorectal, gastroesophageal and chronic lymphocytic leukaemia). Between September 2008 and December 2013, subjects with 20–85 years and a histologically confirmed newly diagnosed colon or rectum cancer (ICD-10: C18, C19, C20, D01.0, D01.1, D01.2) were recruited in 23 Spanish hospitals. Population-based controls frequency-matched to cases by age (same 5-year interval), sex and region with the join distribution of the tumours included in MCC-Spain were randomly selected from primary care centres within hospitals' catchment areas. Participation rates among CRC cases and controls were 68 and 53%, respectively.

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All participants signed an informed consent prior to their inclusion in the study. The study has been approved by the ethics committees of all participating centres and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Additional information regarding the study design is provided elsewhere [24].

Data collection

A computerized epidemiological questionnaire including self-reported socio-demographic and anthropometric data, family history of cancer, environmental exposures, smoking habits, use of selected drugs, reproductive history and current and past lifestyle behaviours (including leisure time physical activity and sedentary lifestyle) was administered by trained personnel in face-to-face interviews at enrolment [24]. Waist and hip circumferences were measured by the interviewer [24].

Subjects were provided a self-administered, validated, semi-quantitative, 140-food item, Spanish Food Frequency Questionnaire (FFQ) [25], modified to include regional products, meat cooking methods and pictures to establish doneness preference (response rate 88%). The FFQ assessed usual dietary intake during the previous year. Food composition was obtained using data from the Centre for Higher Studies in Nutrition and Dietetics (CESNID) and other specific sources [26]. Cross-check questions [27, 28] on food groups intakes were used to adjust the frequency of foods eaten and reduce misreporting of food groups with large numbers of items [25, 26]. Further assessment of misreporting included the identification of potential under- and over-reporting subjects using the predicted total energy expenditure (pTEE) method [29]. pTEE was estimated using Dietary Reference Intakes prediction equations, and implausible reporters were identified on the basis of the ratio of reported intakes to estimated requirements (rEI:pTEE).

Frequency data were used to derive amount (g/day, g/1000 kcal) of each of the individual meat types and summary variables. Meat-related variables were grouped as follows: (1) red meat included all types of beef, pork, lamb and duck; (2) white meat included chicken, turkey and rabbit; (3) organ meat included all types of liver and offal; (4) cured meat included cooked ham, Spanish cured ham, bacon and other Spanish cured sausages (*chorizo*, *fuet*, *salchichón* and others); (5) processed meat included all types of hamburgers and to-be-cooked sausages, hot dogs, meat balls and other meat products. Doneness preference was grouped into three categories: rare, medium and well done. Cooking methods were grouped into four non-exclusive categories: griddle-grilling/ barbequing, pan-frying/ bread-coated frying, stewing, and oven-baking/ others.

A total of 2140 CRC cases and 3950 controls were eligible for this study. We excluded participants with missing dietary data, extreme daily caloric intake (top and bottom 1% of reported energy intake), unreliable anthropometry or missing information in covariates of interest. More information on exclusion criteria can be found in the Online Supporting Material (OSM Fig. 1).

Statistical analyses

Main variables were described using n (%) or mean (SD), and differences assessed using Chi-squared test or *t* test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using two unconditional logistic regression models comparing sex-specific meat intake tertiles defined in all population, or categories of doneness preference or cooking methods. Gender-specific models were built and interaction tests performed. Model 1 included age, gender, area and educational level (less than primary school; primary school; secondary school; university). Model 2 corresponded to a fully adjusted model including age, area, gender, educational level, body mass index (BMI) one year before recruitment, total energy intake, plausibility of reported intake, smoking status one year before recruitment (never; former; current), physical activity during the previous 10 years excluding 2 years before recruitment (inactive; moderately active; active; very active), intakes of fruits, vegetables, nuts, dairy products and fibre, alcohol intake at age 30–40, family history of CRC, use of anti-inflammatory drugs, use of hormone replacement therapy (only in women models) and season of FFQ administration. Meat doneness models were further adjusted by the total intake of the corresponding meat group. The food density method was used for energy adjustment (grams of meat/1000 kcal per day). Goodness of fit was assessed using the Hosmer–Lemeshow test [30]. Sensitivity analyses using multivariate mixed models were fitted including area as a random effect variable, or excluding potential over-/under-reporting subjects. Data analysis was conducted using Stata 12.1 (StataCorp, College Station, TX, USA).

Results

After applying exclusion criteria, a total of 1671 CRC cases and 3095 controls were included in the analyses. There were no noteworthy differences in the characteristics of included and excluded subjects. Socio-demographic, lifestyle and key dietary characteristics of included cases and controls are shown in Table 1. Controls tended to be younger, more physically active and with a higher educational level. Table 2 shows meat intake, doneness preferences and cooking methods. In men, cases consistently

Table 1 Socio-demographic and lifestyle characteristics in the MCC-Spain study

	Controls ($n = 3095$) n (%) or mean (SD)	Cases ($n = 1671$) n (%) or mean (SD)	p value
Gender: Males	1642 (53%)	1092 (65%)	<0.001
Age (years)	63 (12)	67 (11)	<0.001
Education			<0.001
Less than primary school	519 (17%)	515 (31%)	
Primary school	985 (32%)	642 (38%)	
Secondary school	916 (29%)	342 (21%)	
University	675 (22%)	172 (10%)	
BMI (kg/m^2)[a]			<0.001
Underweight	31 (1%)	9 (0%)	
Normal weight	1169 (38%)	482 (29%)	
Overweight	1308 (42%)	768 (46%)	
Obese	587 (19%)	412 (25%)	
Smoking status[a]			<0.001
Never	1324 (43%)	664 (40%)	
Former	1118 (36%)	707 (42%)	
Current	653 (21%)	300 (18%)	
Physical activity[b]			<0.001
Inactive	1185 (38%)	853 (51%)	
Moderately active	457 (15%)	186 (11%)	
Active	382 (12%)	146 (9%)	
Very active	1071 (35%)	486 (29%)	
Anti-inflammatory therapy	949 (31%)	317 (19%)	<0.001
Hormone replacement therapy	108 (3%)	25 (2%)	<0.001
Total energy (kcal/day)	1893 (556)	1987 (607)	<0.001
Current alcohol intake (g/day)	10.9 (16)	12.2 (19)	0.012
Past alcohol intake (g/day)[c]	17.4 (27)	24.2 (34)	<0.001
Calcium (mg/day)	912 (310)	909 (323)	0.730
Fibre (g/day)	1.21 (0.4)	1.14 (0.4)	<0.001
Fruits (g/day)	348 (214)	342 (198)	0.301
Vegetables (g/day)	188 (119)	175 (110)	<0.001
Nuts and seeds (g/day)	11 (22)	9 (18)	<0.001
Fish (g/day)	64 (37)	65 (37)	0.699
Dairy products (g/day)	314 (180)	301 (174)	0.015

[a] One year before recruitment; numbers do not sum up due to missing values

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

[c] Alcohol intake at age 30–40

reported having higher intakes of all meat categories and a preference for a higher degree of meat doneness. There were no differences in meat intakes in women, but cases consistently reported a preference for a higher degree of red meat doneness.

Table 3 shows associations between meat intake and CRC in men and women. Overall, high total meat intake was associated with increased risk of CRC (OR_{T3-T1} 1.41; 95% CI 1.19–1.67; $p_{trend} < 0.001$). White meat (OR_{T3-T1} 1.24; 95% CI 1.05–1.47; p_{trend} 0.012) and red meat (OR_{T3-T1} 1.28; 95% CI 1.09–1.51; p_{trend} 0.003) were associated with increased CRC risk, while the association for processed/cured/organ meat was borderline significant (OR_{T3-T1} 1.17; 95% CI 0.99–1.39; p_{trend} 0.063). These associations were observed for total, processed and red meat in men, but only for white meat in women. Associations were stronger in men than in women. However, a test for interaction was not statistically significant and these differences could be attributed to the smaller number of women in the study. Results according to tumour location are shown in the online supplement (Table 1). Briefly, the association between total meat intake and colon tumours (OR_{T3-T1} 1.30; 95% CI

Table 2 Meat intake, meat doneness preference and meat cooking methods in the MCC-Spain study

Daily intake	Men			Women		
	Controls ($n = 1642$)	Cases ($n = 1092$)	p value	Controls ($n = 1453$)	Cases ($n = 579$)	p value
	n (%) or mean (SD)	n (%) or mean (SD)		n (%) or mean (SD)	n (%) or mean (SD)	
White meat						
Non-consumers	89 (5%)	53 (5%)	0.513	76 (5%)	24 (4%)	0.307
Intake (g/day)	23 (17)	26 (19)	<0.001	20 (15)	21 (14)	0.200
Red meat						
Non-consumers	75 (5%)	44 (4%)	0.499	91 (6%)	44 (8%)	0.275
Intake (g/day)	34 (26)	41 (31)	<0.001	25 (20)	24 (19)	0.135
Processed meat[a]						
Non-consumers	20 (1%)	8 (1%)	0.217	26 (2%)	10 (2%)	0.923
Intake (g/day)	38 (27)	45 (34)	<0.001	27 (21)	28 (20)	0.656
Total meat						
Non-consumers	10 (1%)	4 (0%)	0.384	17 (1%)	7 (1%)	0.941
Intake (g/day)	95 (46)	111 (57)	<0.001	72 (38)	72 (35)	0.977
Doneness preference	n (%)	n (%)	p value	n (%)	n (%)	p value
White meat						
Rare	115 (8%)	44 (4%)		70 (5%)	24 (5%)	
Medium	889 (62%)	580 (60%)		859 (65%)	318 (61%)	
Well-done	436 (30%)	349 (36%)	<0.001	389 (30%)	180 (34%)	0.110
Red meat						
Rare	171 (11%)	72 (7%)		154 (12%)	29 (6%)	
Medium	1054 (70%)	736 (73%)		897 (69%)	351 (68%)	
Well-done	290 (19%)	200 (20%)	0.003	256 (19%)	132 (26%)	<0.001
Cooking methods[b]	n (%) or mean (SD)	n (%) or mean (SD)	p value	n (%) or mean (SD)	n (%) or mean (SD)	p value
White meat						
Griddle-grilled/barbecued						
Non-consumers	463 (30%)	289 (28%)	0.272	293 (21%)	136 (25%)	0.123
Intake (g/day)	9.5 (8.7)	9.7 (8.8)	0.576	8.7 (8.3)	9.1 (8.7)	0.451
Pan-fried/bread-coated fried						
Non-consumers	598 (39%)	393 (38%)	0.726	545 (40%)	223 (40%)	0.807
Intake (g/day)	7.1 (6.2)	7.5 (8.4)	0.311	5.9 (5.4)	5.9 (4.7)	0.918
Stewed						
Non-consumers	382 (25%)	196 (19%)	0.001	294 (21%)	105 (19%)	0.232
Intake (g/day)	7.6 (7.7)	8.4 (8.2)	0.034	6.4 (6.2)	7.0 (7.7)	0.087
Oven-baked/other						
Non-consumers	568 (37%)	348 (34%)	0.108	465 (34%)	210 (38%)	0.090
Intake (g/day)	5.4 (5.8)	5.6 (5.5)	0.675	4.9 (5.2)	4.4 (3.6)	0.107
Red meat						
Griddle-grilled/barbecued						
Non-consumers	187 (12%)	117 (11%)	0.547	133 (10%)	65 (12%)	0.126
Intake (g/day)	17.9 (15.5)	20.1 (16.6)	<0.001	13.6 (10.8)	13.5 (11.6)	0.865
Pan-fried/bread-coated fried						
Non-consumers	518 (33%)	332 (32%)	0.461	489 (36%)	216 (40%)	0.070
Intake (g/day)	13.3 (13.5)	15.8 (15.4)	<0.001	9.7 (9.7)	8.9 (8.7)	0.191
Stewed						
Non-consumers	360 (23%)	227 (22%)	0.430	357 (26%)	129 (24%)	0.346
Intake (g/day)	10.1 (9.8)	12.4 (14.1)	<0.001	7.5 (6.8)	8.5 (11.2)	0.037

Table 2 continued

Cooking methods[b]	*n* (%) or mean (SD)	*n* (%) or mean (SD)	*p* value	*n* (%) or mean (SD)	*n* (%) or mean (SD)	*p* value
Oven-baked/other						
Non-consumers	704 (45%)	499 (48%)	0.177	646 (47%)	312 (58%)	<0.001
Intake (g/day)	5.4 (6.6)	6.2 (6.8)	0.034	4.4 (4.6)	3.9 (4.4)	0.150

Differences assessed using Chi-squared test or *t* test as appropriate. All *p* values are unadjusted. Mean intakes include non-consumers

[a] Including processed meat, cured meat and organ meat

[b] Cooking methods are non-exclusive (each subject could report using more than one method)

Table 3 OR (95% CI) for the associations between sex-specific tertiles of relative meat intake (g/1000 kcal) and colorectal cancer in the MCC-Spain study

	All (1671 cases/3095 controls)		Men (1092 cases/1642 controls)		Women (579 cases/1453 controls)	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
White meat						
*T*1	Ref	Ref	Ref	Ref	Ref	Ref
*T*2	1.08 (0.92–1.25)	1.17 (0.99–1.37)	0.99 (0.82–1.21)	1.06 (0.86–1.31)	1.20 (0.93–1.55)	1.33 (1.00–1.76)
*T*3	1.08 (0.92–1.26)	1.24 (1.05–1.47)	1.04 (0.86–1.27)	1.23 (0.99–1.51)	1.18 (0.91–1.53)	1.35 (1.01–1.80)
P_{trend}	0.357	0.012	0.671	0.062	0.232	0.048
Red meat						
*T*1	Ref	Ref	Ref	Ref	Ref	Ref
*T*2	1.16 (0.99–1.35)	1.12 (0.96–1.32)	1.15 (0.95–1.41)	1.13 (0.92–1.39)	1.15 (0.90–1.49)	1.09 (0.84–1.42)
*T*3	1.35 (1.16–1.58)	1.28 (1.09–1.51)	1.49 (1.22–1.82)	1.46 (1.18–1.80)	1.15 (0.89–1.49)	1.04 (0.79–1.38)
P_{trend}	<0.001	0.003	<0.001	<0.001	0.290	0.763
Processed meat[a]						
*T*1	Ref	Ref	Ref	Ref	Ref	Ref
*T*2	1.12 (0.96–1.31)	1.02 (0.87–1.20)	1.07 (0.88–1.31)	0.98 (0.79–1.20)	1.20 (0.93–1.55)	1.12 (0.86–1.47)
*T*3	1.37 (1.17–1.61)	1.17 (0.99–1.39)	1.42 (1.16–1.73)	1.24 (1.00–1.55)	1.31 (1.01–1.71)	1.11 (0.83–1.47)
P_{trend}	<0.001	0.063	0.001	0.047	0.041	0.482
Total meat						
*T*1	Ref	Ref	Ref	Ref	Ref	Ref
*T*2	1.17 (1.00–1.36)	1.14 (0.97–1.34)	1.16 (0.95–1.41)	1.12 (0.91–1.39)	1.20 (0.93–1.55)	1.18 (0.90–1.55)
*T*3	1.47 (1.25–1.72)	1.41 (1.19–1.67)	1.54 (1.26–1.89)	1.53 (1.23–1.90)	1.38 (1.06–1.80)	1.32 (0.99–1.76)
P_{trend}	<0.001	<0.001	<0.001	<0.001	0.015	0.056

Tertiles of meat intake (g/1000 kcal) defined as follows. In men: white meat ≤8.0, >8.0–13.6, >13.6; red meat ≤11.7, >11.7–21.2, >21.2; processed meat ≤12.7, >12.7–22.5, >22.5; and total meat ≤38.9, >38.9–57.3, ≥57.3. In women: white meat ≤7.8, >7.8–13.3, >13.3; red meat ≤8.9, >8.9–16.8, >16.8; processed meat ≤10.1, >10.1–17.9, >17.9; and total meat ≤32.4, >32.4–48.4, >48.4

Model 1 adjusted by age, area, gender (only in "All" model) and educational level

Model 2 adjusted by age, area, gender (only in "All" model), BMI one year before recruitment, educational level, total energy intake, plausibility of reported intake, smoking status one year before recruitment, physical activity during the previous 10 years (excluding 2 years before recruitment), intakes of: fruits, vegetables, nuts, dairy products and fibre, alcohol intake at age 30–40, family history of colorectal cancer, use of anti-inflammatory drugs, use of hormone replacement therapy (only in women) and season of FFQ

[a] Including processed meat, cured meat and organ meat

1.04–1.63; p_{trend} 0.024) was weaker than in rectum tumours (OR_{T3-T1} 1.67; 95% CI 1.20–2.31; p_{trend} 0.003). Furthermore, associations according to meat subtypes were only significant in rectum tumours: red meat (OR_{T3-T1} 1.53; 95% CI 1.13–2.08; p_{trend} 0.005) and processed meat (OR_{T3-T1} 1.38; 95% CI 1.00–1.89; p_{trend} 0.048).

The association between meat doneness preference and CRC is shown in Table 4. No overall differences in CRC risk were observed when comparing preference for well-done meat versus medium doneness in white, red or total meat. However, preference for rare meat doneness was associated with a significant reduction in CRC risk in red

Table 4 OR (95% CI) for the associations between meat doneness preference and colorectal cancer in the MCC-Spain study

Meat doneness	All		Men		Women	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
White meat						
Rare	0.68 (0.50–0.92)	0.77 (0.56–1.05)	0.62 (0.43–0.91)	0.69 (0.47–1.02)	0.82 (0.49–1.37)	0.99 (0.58–1.70)
Medium	Ref	Ref	Ref	Ref	Ref	Ref
Well-done	1.05 (0.91–1.22)	1.04 (0.90–1.22)	1.07 (0.89–1.30)	1.07 (0.88–1.31)	1.04 (0.81–1.32)	1.07 (0.82–1.38)
P_{trend}	0.041	0.146	0.034	0.084	0.494	0.650
Red meat						
Rare	0.63 (0.50–0.81)	0.66 (0.51–0.85)	0.67 (0.50–0.90)	0.68 (0.49–0.93)	0.58 (0.37–0.90)	0.63 (0.39–1.00)
Medium	Ref	Ref	Ref	Ref	Ref	Ref
Well-done	0.90 (0.76–1.07)	0.91 (0.77–1.09)	0.79 (0.63–0.98)	0.80 (0.64–1.01)	1.16 (0.88–1.52)	1.10 (0.83–1.47)
P_{trend}	0.151	0.211	0.964	0.986	0.013	0.070
Total meat						
Rare	0.55 (0.37–0.82)	0.56 (0.37–0.86)	0.60 (0.37–0.97)	0.58 (0.35–0.97)	0.44 (0.21–0.94)	0.48 (0.22–1.05)
Medium	Ref	Ref	Ref	Ref	Ref	Ref
Well-done	0.99 (0.82–1.19)	1.03 (0.85–1.26)	0.82 (0.64–1.05)	0.85 (0.66–1.11)	1.27 (0.95–1.70)	1.31 (0.96–1.78)
P_{trend}	0.148	0.094	0.795	0.934	0.011	0.013

Model 1 adjusted by age, area, gender (only in "All" model) and educational level

Model 2 adjusted by age, area, gender (only in "All" model), BMI one year before recruitment, educational level, total energy intake, plausibility of reported intake, smoking status one year before recruitment, physical activity during the previous 10 years (excluding 2 years before recruitment), intakes of: the corresponding meat group, fruits, vegetables, nuts, dairy products and fibre, alcohol intake at age 30–40, family history of colorectal cancer, use of anti-inflammatory drugs, use of hormone replacement therapy (only in women) and season of FFQ

Non-consumers were excluded from the analyses

($OR_{rare\ vs.\ medium}$ 0.66; 95% CI 0.51–0.85) and total meat ($OR_{rare\ vs.\ medium}$ 0.56; 95% CI 0.37–0.86). Overall, only women showed significant trends towards increased risk according to higher meat doneness preferences (total meat: p_{trend} 0.013).

As shown in Table 5, CRC risk varied by cooking method. An above-the-median intake of griddle-grilled or barbecued meat was associated with an increased CRC risk when comparing to non-consumers (OR 1.45; 95% CI 1.13–1.87). Similarly, stewed or oven-baked white, but not red, meat was associated with increased CRC risk (stewed: OR 1.25; 95% CI 1.04–1.51; oven-baked: OR 1.18; 95% CI 1.00–1.40). On the other hand, a moderate consumption (below the median) of pan-fried/bread-coated fried or oven-baked/other red meat was associated with a reduction in CRC risk (pan-fried/bread-coated fried: OR 0.81; 95% CI 0.68–0.96; oven-baked/other: OR 0.77; 95% CI 0.65–0.91). No other differences in CRC risk were found for other meat cooking methods.

Sensitivity analyses excluding potential over-/under-reporting subjects yielded very similar results. No differences were found when comparing the results of multivariate mixed models including area as a random effect variable to logistic regression models adjusted for area (data not shown).

Discussion

The results of our case–control study including 1671 CRC cases and 3095 controls support an association between meat consumption, and meat cooking practices, and the risk of CRC. Specifically, white, red, processed/cured/organ and total meat intakes were associated with increased risk of CRC. However, red meat was more strongly associated with CRC in men than in women, whereas white meat showed stronger associations in women than in men. Regarding meat cooking practices, we found that rare-cooked meat preference was associated with low risk of CRC when considering red and total meat, although trends towards increased risk according to higher meat doneness preferences were only significant in women. Finally, our results suggest that griddle-grilling and barbequing could be associated with increased CRC risk in all kinds of meat, whereas stewing and oven-baking could increase the risk with respect to white, but not red, meat.

Our finding of an association between meat intake and CRC risk is consistent with the conclusions of the 2011 WCRF/AICR Continuous Update Project Report Summary for Colorectal Cancer [4] and the 114th IARC Monograph [10]. Although recent studies, such as Di Maso 2013 [15] provided similar results, the Multiethnic Cohort Study [31],

Table 5 OR (95% CI) for the associations between meat cooking methods and colorectal cancer in the MCC-Spain study

Cooking methods	OR (95% CI)[a]	p_{trend}
White meat		
Griddle-grilled/barbecued		
No intake	Ref	0.007
Intake below median (≤7 g/day)	1.16 (0.97–1.39)	
Intake above median (>7 g/day)	1.30 (1.08–1.56)	
Pan-fried/bread-coated fried		
No intake	Ref	0.919
Intake below median (≤5 g/day)	0.99 (0.84–1.17)	
Intake above median (>5 g/day)	0.99 (0.84–1.17)	
Stewed		
No intake	Ref	0.025
Intake below median (≤5 g/day)	1.20 (1.00–1.45)	
Intake above median (>5 g/day)	1.25 (1.04–1.51)	
Oven-baked/other		
No intake	Ref	0.054
Intake below median (≤4 g/day)	1.15 (0.97–1.36)	
Intake above median (>4 g/day)	1.18 (1.00–1.40)	
Red meat		
Griddle-grilled/barbecued		
No intake	Ref	0.008
Intake below median (≤13 g/day)	1.00 (0.79–1.26)	
Intake above median (>13 g/day)	1.24 (0.98–1.57)	
Pan-fried/bread-coated fried		
No intake	Ref	0.503
Intake below median (≤8 g/day)	0.81 (0.68–0.96)	
Intake above median (>8 g/day)	0.95 (0.80–1.12)	
Stewed		
No intake	Ref	0.602
Intake below median (≤7 g/day)	0.98 (0.82–1.17)	
Intake above median (>7 g/day)	1.04 (0.87–1.25)	
Oven-baked/other		
No intake	Ref	0.218
Intake below median (≤3 g/day)	0.77 (0.65–0.91)	
Intake above median (>3 g/day)	0.94 (0.79–1.11)	
Total meat		
Griddle-grilled/barbecued		
No intake	Ref	0.002
Intake below median (≤22 g/day)	1.25 (0.98–1.59)	
Intake above median (>22 g/day)	1.45 (1.13–1.87)	
Pan-fried/bread-coated fried		
No intake	Ref	0.911
Intake below median (≤12 g/day)	0.86 (0.72–1.02)	
Intake above median (>12 g/day)	0.98 (0.81–1.18)	
Stewed		
No intake	Ref	0.119
Intake below median (≤11 g/day)	1.13 (0.91–1.40)	
Intake above median (>11 g/day)	1.20 (0.96–1.49)	
Oven-baked/other		
No intake	Ref	0.444
Intake below median (≤6 g/day)	1.01 (0.85–1.20)	
Intake above median (>6 g/day)	1.07 (0.89–1.27)	

[a] Adjusted by age, area, gender, BMI one year before recruitment, educational level, total energy intake, plausibility of reported intake, smoking status one year before recruitment, physical activity during the previous 10 years (excluding 2 years before recruitment), intakes of: fruits, vegetables, nuts, dairy products and fibre, alcohol intake at age 30–40, family history of colorectal cancer, use of anti-inflammatory drugs, use of hormone replacement therapy (only in women) and season of FFQ

an Australian case–control study [18] or the most recent results by Joshi et al. [23] did not support an association between meat and CRC. Moreover, associations for processed meat, but not for red or total meat, were found in a cohort of Norwegian women [16]. Interestingly, all studies reporting no significant associations observed lower meat intakes than our study, and subjects in their high meat intake groups had intakes below the mean meat intake reported in our study (fifth quintile total meat median = 69g/1000 kcal per day [31]; only 5% of the sample having more than 35g/day of red meat [16]). This fact suggests that the relationship between meat intake and CRC risk could be non-existing under a certain meat intake threshold and become apparent with higher meat intake, thus explaining part of the heterogeneity of results in the literature. This fact, together with the smaller number of females in our study, could partially explain the weaker results found in women, who reported lower intakes of red, processed and overall meat than in men. It is also noteworthy that the relationship between meat intake and CRC was much stronger when the tumour was located in the rectum than in the colon. A 2006 meta-analysis stated that the association between red meat and rectal tumours was stronger than with colon tumours [32], and other studies have confirmed this risk difference according to tumour subsite [15, 17]. Finally, reported findings on white meat were unexpected. According to the most recent meta-analysis, poultry intake could be moderately associated with reduced incidence of CRC, although there is heterogeneity in available study results [33]. In this sense, it is noteworthy that the daily intake of white meat in our cohort was high 20–26 g per day and thus higher than most studies included in previous meta-analyses. This would favour the hypothesis of a non-existing relationship between meat intake and CRC under a certain meat intake threshold. Another potential explanation could involve population differences in susceptibility [34]. In any case, further research is needed to determine reasons for heterogeneity in study results.

Although it has been suggested that high-temperature meat cooking methods with direct contact with the heat source could further increase the risk of CRC, the results of studies considering cooking methods and meat doneness have not been consistent [15–23]. In our study, we found that red and total meat rare-cooked preference was associated with lower risk of CRC when comparing to medium-cooked preference, but no significant differences in risk were found when comparing medium with well-done meat preference. Interestingly, results for meat doneness preference were stronger in women than in men. The cause of these differences is unknown, although it could partially be explained by differences in meat doneness reporting accuracy. As an alternative hypothesis, cooking preference effect could be shadowed by high meat intake, thus being more evident in women as they usually have lower intakes of meat than in men. Differences in both cooking habits and total amount of meat consumed, together with genotypic heterogeneity among populations, could partly explain the lack of consistency among the different studies results. As initially hypothesized, griddle-grilling or barbecuing was associated with increased CRC risk in all kinds of meat. Interestingly, we also found stewing and oven-baking to increase the risk of white, but not red, meat. Traditional Spanish stewing is usually cooked in two phases: first the meat is browned at high temperature and then cooked for a long time at low temperature. We hypothesize that carcinogenic compounds produced during the browning phase would remain in the sauce during the second phase, thus increasing the risk of CRC. However, this mechanism would not explain risk differences in red and white meat. This same principle could contribute to explain the null, or even negative, results in subjects with intakes below the median, found for pan-frying or bread-coated frying, as the carcinogenic compounds would remain in the oil, which is removed before serving. In this regard, the characteristics of frying practices in Spain should be noted, as there is an almost exclusive use of olive and sunflower oils, which are less prone to oxidation than other fats, and oil is not reused many times at home [35].

Different biological mechanisms have been suggested to explain the relationship between meat intake and CRC. It is known that haem iron from red meat can increase the exposure to carcinogenic NOCs [11–13]. Recent results highlight the role of haem iron in the promotion of colon cancer by red meat and further suggest that haem iron could initiate carcinogenesis through lipid peroxidation [36]. Similarly, nitrites and nitrates added to meat for preservation could further increase the exogenous exposure to NOCs [13]. Moreover, high red meat intake has been reported to increase the expression of oncogenic microRNA in the rectal mucosa [37]. The involvement of bovine viruses has also been suggested [38]. Finally, HCAs and PAHs formed during high-temperature meat cooking have mutagenic and carcinogenic properties [11, 14].

Our study has several strengths. All participants answered a questionnaire covering established and suspected risk factors for CRC in addition to a FFQ including data on meat cooking practices. The number of subjects was sufficiently large to detect differences not only in meat intake but also in cooking methods. The multicentric design allowed for a wide geographic variability, ensuring a sufficient heterogeneity of intakes and cooking practices. Finally, all tumours were newly diagnosed and histologically confirmed, and information on tumour location was available. On the other hand, limitations in the current study include: (1) the case–control design imply the use of recent dietary data; (2) use of self-reported data, thus subjected to recall bias; (3) measurement error in the estimation of meat intake due to the use of a FFQ, though it has been previously used in Spanish population and adapted to include regional products [25]; (4) the selection of controls was performed according to the joint distribution of all tumours included in the MCC-Spain study in each site; thus, the characteristics of each site's set of controls were highly dependent on the tumours recruited in the site. This caused a satisfactory overall matching when considering all tumours together but a suboptimal matching when focussing in a single tumour at a time. To solve this, part of the controls (those most dissimilar to cases of the studied tumour) were excluded, and a basic adjustment set including age, area, gender and educational level was used in all regression models; finally, we cannot exclude (5) potential residual confounding.

In conclusion, our study supports an association of white, red, processed/cured/organ and total meat intake with an increased risk of CRC. When considering meat cooking practices, we reported rare-cooked red and total meat preference to be associated with low risk of CRC among meat consumers. Griddle-grilling and barbequing meat could be associated with increased CRC risk and stewing, and oven-baking could increase the risk of white, but not red, meat. Overall, our results support the recommendation of moderating meat intake, and reducing or avoiding processed meat consumption.

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Compliance with ethical standards

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

Ethical standards All participants signed an informed consent prior to their inclusion in the study. The study has been approved by the ethics committees of all participating centres and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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