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Meat consumption and meat preparation in relation to colorectal adenomas among sporadic and HNPCC family patients in The Netherlands

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Abstract

Meat consumption and meat preparation methods are thought to be associated with the risk of sporadic colorectal cancer, and possibly adenomas. As the same somatic mutations occur in sporadic adenomas and hereditary non-polyposis colorectal cancer (HNPCC)-related adenomas, similar exogenous factors may play a role in the development of both types of adenoma. In a case control study among 57 sporadic colorectal adenoma cases and 62 adenoma cases from HNPCC families (and 148 adenoma-free controls) from the Netherlands, we examined whether meat consumption and preparation are similarly associated with sporadic and suspected HNPCC colorectal adenomas. Frequency of meat consumption was not significantly associated with adenoma risk in our population of sporadic and HNPCC family cases and controls (Odds Ratios (OR) for high versus low consumption were 1.0 and 0.6, respectively). Interestingly, consumption of red meat and specific preparation methods (i.e., "not adding any water" and " closed lid with most meat types") slightly, but non-significantly, increased the risk of adenomas in the sporadic group only (OR, 95% Confidence Interval (CI): 4.1, 0.7–23.0, 2.0, 0.6–6.5 and 2.6, 0.9–7.2, respectively). This is the first study to examine possible differences or similarities in risk factors for sporadic and HNPCC colorectal carcinogenesis. Our results do not provide support for meat consumption as a risk factor for adenoma formation in HNPCC family members. Some characteristics of habitual meat preparation in the Netherlands may, however, increase the risk of sporadic adenomas.

Keywords: Colorectal adenomas; Hereditary; Sporadic; HNPCC; Case control study; Meat; Preparation; Heterocyclic amines

1. Introduction

Colorectal cancer is thought to develop through an adenoma-carcinoma sequence, by accumulation of alterations in genes that control cell growth and differentiation [1]. This accumulation of genetic alterations and the progression to cancer is thought to be promoted by diets high in fat and/or meat and low in fibre and/or fruits and vegetables [2].

Hereditary non-polyposis colorectal cancer (HNPCC) is a syndrome with an autosomal dominant inheritance pattern, caused by a germline mutation in one of the mismatch repair genes, resulting in microsatellite instability (MSI) [3]. HNPCC mutation carriers develop colorectal adenomas in a similar manner as sporadic patients. However, characteristic of HNPCC is a very rapid progression from adenoma to carcinoma, possibly due to the defective mismatch repair system. Besides colorectal cancer, HNPCC mutation carriers have a higher risk of developing malignancies at several other sites. Interestingly, the tumour spectrum in HNPCC has changed over generations [4], suggesting a role for

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environmental factors in HNPCC carcinogenesis. Other characteristics of HNPCC (e.g. incomplete penetrance [5], earlier onset of disease in successive generations [6–8], somatic mutations in *APC*, K-*ras* and *TP*53 genes [9,10–12]) point in the same direction. It is possible that the environmental factors relevant in sporadic colorectal carcinogenesis also play a role in HNPCC-related colorectal adenoma development.

Several cohort studies and case-control studies have suggested that a high intake of (red) meat may be associated with the risk of sporadic colorectal cancer, independent of the fat content of the diet (for reviews see [13–15]). An increased risk of colorectal adenomas is associated with a high consumption of red meat in some studies [16–18], but not in others [19,20]. During the preparation of meat at high temperatures heterocyclic amines may be formed, which have been found to be mutagenic and cause tumours in the colon and various other organs in animal models [21]. Several studies have found the increased risk of colorectal carcinomas or adenomas to be specifically associated with fried meat [22], grilled/barbecued meat [23], high temperature cooking of meat [24], 'well done' meat [23], meat with a heavily browned surface [24], or a combination of factors related to meat consumption and meat preparation [25]. However, results are inconsistent and remain inconclusive (for reviews see [13–15]).

This case-control study examines whether meat consumption and meat preparation habits in the Netherlands are similarly related to the risk of sporadic and suspected HNPCC colorectal adenomas.

2. Patients and methods

2.1. Study population

This case-control study on dietary factors and genetic susceptibility in colorectal adenomas was conducted in The Netherlands between December 1995 and February 1998, and approved by the medical ethical committees of the Wageningen University and the University Medical Center St. Radboud. The study population included sporadic adenoma cases as well as adenoma cases from HNPCC families, and control-groups for each case group. All participants were Dutch-speaking and of Western European origin, diagnosed before the age of 75 years, without a history of colorectal cancer, colon resection, polyposis coli, or inflammatory bowel disease.

Cases and controls from HNPCC families were recruited either by the University Medical Center St. Radboud or by the Netherlands Foundation for the Detection of Hereditary Tumors which keeps a registry of HNPCC families in The Netherlands, and were all examined for screening purposes. The pedigrees of all HNPCC families fulfilled the Amsterdam criteria [26] (i.e., at least three members with colorectal cancer in at least two successive generations, with at least one case diagnosed before the age of 50 years; one of the affected members was a first-degree relative of the other two; familial adenomatous polyposis was ruled out). All cases and controls from these HNPCC families were first-degree relatives of colorectal or endometrium cancer cases. For most families, mutation status of 'HNPCC-genes' was unknown, therefore HNPCC cases and controls were assumed to have a theoretical 50% chance of being a mutation-carrier.

The HNPCC controls had never been diagnosed with colorectal adenomas or cancer, and had a full colonic examination (colonoscopy or sigmoidoscopy combined with barium enema) to exclude the presence of adenomas. Cases from HNPCC families were allowed to have had adenomas previously, as this increased the efficiency of the study.

Sporadic cases and controls were recruited by the departments of Gastroenterology of the University Medical Center St. Radboud and two regional hospitals (Rivierenland, Tiel; Gelderse Vallei, Ede), and were examined at the hospital for various reasons such as bowel complaints (abdominal pain, rectal blood loss, constipation, diarrhoea). Controls had never been diagnosed with colorectal adenomas or cancer, and had a full colonic examination (colonoscopy or sigmoidoscopy combined with barium enema) to exclude the presence of adenomas. Sporadic cases were excluded if they had previous adenomas more than 3 years before their entrance into the study, as a long history of repeated endoscopies and colorectal adenomas may be related to possible changes in dietary habits in these patients.

2.2. Subject recruitment and data collection

Eligible subjects were informed about the study by the gastroenterologist participating in the study or by the Netherlands Foundation for the Detection of Hereditary Tumors, after approval by their 'physician in attendance'. Nine percent of subjects invited by the University Medical Center St. Radboud, and 12% of subjects invited by the Netherlands Foundation for the Detection of Hereditary Tumors, were not willing to participate in the study. In total, 319 eligible subjects signed for informed consent. Subsequently, those who were willing to participate received a set of questionnaires at endoscopy (in case of recruitment by one of the hospitals) or by mail (in case of recruitment by the Netherlands Foundation for the Detection of Hereditary Tumors). The set of questionnaires consisted of a questionnaire on lifestyle and socioeconomic factors, a semi-quantitative food frequency questionnaire and a questionnaire on meat consumption and preparation. The questionnaires were filled out at home and returned by mail.

Case/control status and HNPCC/sporadic status were confirmed based on hospital records retrospectively, independently of exposure status, after we received the filled-out questionnaires and informed consent. Of the 319 patients who initially agreed to participate in the study 22 (7%) did not return the questionnaires, and 21 (7%) did not meet the eligibility criteria retrospectively. Additionally, 9 participants filled out the meat consumption and preparation questionnaire incompletely and were therefore excluded from the present analyses, resulting in 267 subjects available for further study. Data entry of the questionnaires was performed by those blinded to disease status.

Of the 57 sporadic adenoma cases, 8 had been diagnosed with previous adenomas on average 2.5 years before the current index adenoma for which they were included in the study. Among the 62 'suspected HNPCC' adenoma cases, 12 patients had been diagnosed with previous adenomas on average 4 years before the current index adenoma for which they were included in the study. The control groups consisted of 65 sporadic and 83 'suspected HNPCC' controls. As mentioned above, for most HNPCC families, mutation status of 'HNPCC genes' was unknown. However, 2 adenoma cases and 5 controls were known to be 'non-carriers' and were therefore included in the sporadic group. In the HNPCC group, 2 cases and 10 controls were known to be 'mutation carriers' at the time this study was conducted.

2.3. Dietary assessment

To quantify energy, nutrient intake and meat consumption a validated semi-quantitative food frequency questionnaire was used, which was developed for the Dutch part of the EPIC study (European Prospective Investigation into Cancer and Nutrition) [27]. Frequency of consumption of food groups was based on the habitual consumption of 178 food items during the year before endoscopy. Nutrient intake was quantified for each individual using an extended version of the 1993 computerised Dutch food composition table.

To obtain additional information on the consumption and preparation of red meat and poultry, a questionnaire was developed especially for this purpose. The questionnaire consists of four parts, starting with some general questions regarding frequency of eating a hot meal, meat with the hot meal, grilled or barbecued meat, and gravy, as well as a question on preference with respect to colour of the meat surface. The second part focuses on the frequency and portion size of 16 types of meat. Intensity of surface browning of four types of meat (beef patties, pork chops, steak and bacon) was assessed using four photos for each type of meat, showing the meat with varying degrees of surface browning (from very dark to very light, prepared at 225, 200, 175 and 150 °C, respectively). The photos originate from a Swedish questionnaire used in a case-control study on intake of heterocyclic amines in relation to cancer risk [28]. In the last part of our questionnaire, preparation methods for each of six meat categories were assessed using multiple choice questions on factors relevant to the temperature during meat preparation: type of pan used, temperature of cooking fat when meat is added, intensity of the heat source, addition of water, use of a lid, surface browning at the end stage of cooking compared with just after the browning phase.

2.4. Data analysis

Differences in general characteristics between groups were tested using χ^2-tests for categorical and *t*-tests for continuous variables. All analyses were stratified for the HNPCC families and the sporadic group. Multivariate logistic regression analyses were used to estimate the relative risk for adenomas and simultaneously adjust for possible confounding factors.

Odds ratios were calculated for tertiles of nutrients based on the distribution in controls, as well as using the continuous variable and expressing the odds ratio for quartile ranges. Associations with total meat (red meat and poultry), red meat (pork, beef, veal, lamb, game, organs, etc.), white meat (poultry, fish), and gravy were assessed for frequency categories as well as using a test for trend. To take individual habitual meat preparation practices into account, subjects were categorised based on multiple choice questions regarding intensity of the heat source, use of a lid, addition of water (for 6 types of meat), and preferred colour of the meat surface after preparation (4 photo-questions). All possible answers were scored as 'low risk' or 'high risk' and subjects were categorised as 'low risk method for all meat types', 'high risk method for all meat types', or the intermediate category 'method depends on meat type'.

To control for potential confounding by energy intake, all nutrients have been adjusted for energy using the nutrient-residuals from linear regression on energy intake, and additionally, both nutrients and food groups are adjusted for total energy intake by including total energy intake in the logistic regression models. As the age and gender distribution differs between cases and controls and between the HNPCC families and the sporadic group, all analyses were adjusted for age and gender. As the cases in this study may have had a generally 'less healthy' distribution of other lifestyle related risk factors (i.e. higher Body Mass Index (BMI), less never-smokers, a less physically active lifestyle, more regular alcohol drinkers and less regular aspirin/non-steroidal anti-inflammatory drugs (NSAIDs) users), these factors could act as possible confounders, and were separately taken into account by including them in the logistic regression models (in combination with

energy, age, gender). None of the odds ratios markedly changed and the odds ratios for these potential confounders were not statistically significant in the models.

3. Results

General characteristics of the sporadic and HNPCC study populations are shown in Table 1. Mean age was higher in cases than in controls in both groups, and HNPCC cases were younger than sporadic cases. As the HNPCC population regularly had full colonic examinations for screening purposes, the percentage of subjects with bowel complaints was lower than in the sporadic population (Table 1). Among sporadic subjects, 49% of the cases and 62% of the controls reported having bowel complaints. Adenomas diagnosed in HNPCC family members were smaller and more often localised in the proximal colon compared with adenomas in the sporadic group. The two groups did not sig-

Table 1
General characteristics of the sporadic and HNPCC study populations

Variable		Sporadic population		HNPCC population	
		Adenoma cases ($n=57$)	Controls[a] ($n=65$)	Adenoma cases ($n=62$)	Controls[a] ($n=83$)
Demographic data					
Age (years)	mean±SD	54±11	48±13[f]	46±10	39±10[f,g]
Gender	% men	55	38	53	42
Medical characteristics					
Bowel complaints[b]	% yes	49	62	23	24[g]
Family history[c]	% yes	14	23	100	100
Adenoma characteristics[d] (%)					
Adenoma size (mm)	1–4	30		59[h]	
	5–9	28		24	
	⩾10	42		17	
Number of adenomas					
	Single	58		71	
	Multiple	40		27	
Localisation	Distal colon + rectum	79		58[h]	
	Proximal colon	21		42	
Dysplasia	Low grade	36		40	
	Moderate/severe	64		60	
Villocity	Tubular	92		98	
	Tubulovillous/villous	8		2	
Lifestyle characteristics					
Body Mass Index	mean±S.D.	26±3	25±3	26±4	24±3[f,g]
Regular exercise	% yes	58	52	58	62
Smoking	% ex-smoker	48	35	34	27
	% current smoker	22	20	31	27
Alcohol	%⩾1 glass/day	46	38	52	43
Aspirin, NSAIDs	%<1/month	32	28	42	35
Daily macro-nutrient intake[e]					
Energy (MJ)	mean±S.D.	9.6±2.6	9.7±3.1	9.6±2.6	9.5±2.3
Protein (g)	mean±S.D.	89±13	86±15	84±14	84±14
Fat (g)	mean±S.D.	94±20	92±15	95±18	90±15
Carbohydrates (g)	mean±S.D.	128±26	123±29	123±28	126±30

HNPCC, hereditary non-polyposis colorectal cancer. NSAID, non-steroidal anti-inflammatory drugs. S.D., standard deviation.

[a] Controls also underwent full colonic examination.

[b] Bowel complaints in year before endoscopy (data available for 38 sporadic cases, 28 controls, 54 HNPCC cases, 58 controls).

[c] Family history of colorectal cancer in a first-degree family member, percentages exclude 5 controls and 2 cases non-gene-carriers from HNPCC families included in the sporadic group.

[d] Characteristics of the largest adenoma in case of multiple adenomas; data on dysplasia and villocity of adenoma were available for 50 sporadic and 48 HNPCC adenomas.

[e] Nutrients are adjusted for energy intake, using residuals in linear regression.

[f] Statistically significant ($P<0.05$) difference between cases and controls, within the same stratum.

[g] Statistically significant ($P<0.05$) difference between HNPCC and sporadic controls.

[h] Statistically significant ($P<0.05$) difference between HNPCC and sporadic cases.

nificantly differ with respect to multiplicity, dysplasia and villocity of adenomas.

Lifestyle characteristics and macro-nutrient intakes were not significantly different between populations, nor between cases and controls. The only exception was Body Mass Index (BMI), which was significantly lower in HNPCC controls compared with sporadic controls. HNPCC cases had a slightly, but significantly, higher BMI than HNPCC controls. The percentage 'ever smokers', subjects with an average alcohol consumption of one or more glasses per day, and subjects using aspirin or NSAID's once a month or less, were not significantly different between cases and controls in both populations. However, these percentages were always slightly higher in the cases.

Odds ratios of colorectal adenomas were calculated for nutrients that may serve as indicators of meat consumption (Table 2). When analysing tertiles of (animal) protein and fat (or fatty acid) intake no consistent trends were observed. Analysing nutrient intakes as continuous variables, no statistically significant associations were observed, either when cases and controls from both populations were combined (data not shown), or when the sporadic and HNPCC populations were analysed separately (Table 2). There was no evidence for a statistical interaction between dietary factors and HNPCC vs sporadic status (non-significant ORs range between 0.8 and 1.2, data not shown). However, stratification for HNPCC and sporadic groups was maintained for *a priori* considerations.

Total meat intake was not associated with the risk of adenomas in the sporadic as well as the HNPCC group (Table 3). Moreover, no significant association between adenoma risk and red meat intake was observed, although the trend in the sporadic group was in the expected direction and nearly reached statistical significance. For intake of white meat including fish a non-significant inverse association with adenoma risk was observed in the sporadic group only. We found no association between regular consumption of gravy and adenoma risk in either group.

Table 2
"Meat-related" nutrient intakes and the risk of colorectal adenomas in sporadic and HNPCC groups separately

Nutrient intakes[a]			Sporadic population				HNPCC population			
			Controls/cases		OR	(95% CI)	Controls/cases		OR	(95% CI)
Total protein (g)										
	Low	<76	17	9	1.0		31	18	1.0	
	Medium	76–91	27	23	1.3	(0.4–3.8)	24	24	0.9	(0.4–2.4)
	High	> 91	21	25	1.2	(0.3–4.1)	28	20	0.5	(0.2–1.6)
	Continuous	/ 18	65	57	0.9	(0.5–1.6)	83	62	0.7	(0.4–1.2)
Animal protein (g)										
	Low	<45	18	9	1.0		30	15	1.0	
	Medium	45–59	19	31	4.0	(1.3–12.1)	32	31	1.3	(0.5–3.3)
	High	> 59	28	17	0.5	(0.2–1.6)	21	16	1.0	(0.4–2.9)
	Continuous	/ 19	65	57	0.7	(0.4–1.2)	83	62	0.9	(0.5–1.5)
Total fat (g)										
	Low	<83	20	18	1.0		28	15	1.0	
	Medium	83–97	24	16	0.5	(0.2–1.3)	27	20	1.3	(0.5–3.3)
	High	> 97	21	23	0.4	(0.1–1.6)	28	27	1.4	(0.4–4.4)
	Continuous	/ 22	65	57	0.7	(0.4–1.5)	83	62	1.3	(0.7–2.4)
Saturated fatty acids (g)										
	Low	<32	21	19	1.0		27	18	1.0	
	Medium	32–38	22	13	0.4	(0.1–1.2)	29	23	1.0	(0.4–2.4)
	High	> 38	22	25	0.4	(0.1–1.4)	27	21	0.8	(0.2–2.4)
	Continuous	/ 10	65	57	0.8	(0.4–1.5)	83	62	1.0	(0.5–1.8)
N-3 long chain fish fatty acids (g)										
	Low	<0.07	17	22	1.0		31	19	1.0	
	Medium	0.07–0.19	24	16	0.4	(0.1–1.0)	27	22	1.2	(0.5–2.9)
	High	> 0.19	24	19	0.4	(0.2–1.1)	25	21	1.1	(0.4–2.6)
	Continuous	/ 0.14	65	57	0.9	(0.6–1.4)	83	62	0.8	(0.6–1.2)

95% CI, 95% Confidence Interval.

[a] Nutrient intakes are energy adjusted using residual regression analysis, all models are adjusted for age and gender as well as energy intake. Tertile cut-off points are based on the distribution in the "pooled" control group. The odds ratio (OR) for the continuous variables of nutrient intakes are expressed for an exposure contrast representing the difference between the first and third quartile.

Table 3
Meat consumption frequency and the risk of colorectal adenomas in the sporadic and HNPCC group

Consumption frequency			Sporadic population				HNPCC population			
			Controls	Cases	OR	(95% CI)[a]	Controls	Cases	OR	(95% CI)[a]
Total meat (excluding fish)[b]										
	Low	⩽4x /week	8	9	1.0		23	18	1.0	
	Medium	5–6x /week	36	24	0.6	(0.2–2.1)	34	26	0.9	(0.4–2.2)
	High	7x /week	21	24	1.0	(0.3–3.6)	26	18	0.6	(0.2–1.6)
	Trend	*P* value	65	57	$P=0.63$		83	62	$P=0.33$	
Red meat[b]										
	Low	⩽4x /week	19	14	1.0		29	22	1.0	
	Medium	5–6x /week	38	29	1.1	(0.3–3.9)	38	33	1.1	(0.3–3.7)
	High	7x /week	8	14	4.1	(0.7–23.0)	16	7	0.4	(0.1–2.2)
	Trend	*P* value	65	57	$P=0.08$		83	62	$P=0.26$	
White meat (includes fish)[b]										
	Low	⩽0.5x /week	4	9	1.0		15	10	1.0	
	Medium	0.5–2x /week	45	33	0.2	(0.0–0.9)	48	37	1.2	(0.4–3.6)
	High	>2x /week	16	15	0.3	(0.1–1.4)	20	15	1.3	(0.4–4.3)
	Trend	*P*-value	65	57	$P=0.36$		83	62	$P=0.68$	
Gravy										
	Low	⩽2x /week	19	18	1.0		36	22	1.0	
	Medium	⩽2–5x /week	25	17	0.5	(0.2–1.4)	35	23	1.1	(0.5–2.5)
	High	> 5x /week	21	22	0.6	(0.2–1.9)	12	17	1.9	(0.7–5.4)
	Trend	*P* value	65	57	$P=0.38$		83	62	$P=0.26$	

[a] Models are adjusted for age, gender, energy intake and total meat consumption.
[b] Total meat includes red meat and poultry, excludes fish; red meat includes pork, beef, veal, lamb, game, organs etc; white meat includes poultry and fish.

Table 4 shows odds ratios for several characteristic meat preparation methods, which are presumed to reflect the formation of heterocyclic amines. None of these variables were significantly associated with adenoma risk in the sporadic group and the HNPCC families. However, ''not adding water with any meat type'' and ''closed lid with most meat types'' were associated with a non-significant increase in sporadic adenoma risk. Preference for a dark colour of the meat surface, as assessed using photos, was not associated with an increased risk of colorectal adenomas in either group. As the numbers of subjects who preferred a (very) dark meat surface for all meat types or a (very) light meat surface for all meat types were rather small, this resulted in unstable estimates of the Odds Ratio.

4. Discussion

In this study of sporadic and HNPCC-related colorectal adenomas meat consumption and meat preparation were not significantly associated with adenoma risk. Non-significant increases in sporadic adenoma risk with preparation methods that increase cooking temperature and potentially heterocyclic amine formation warrant further research in larger populations of sporadic adenoma cases. No evidence for any association between meat consumption and preparation and adenoma risk in HNPCC families was found. The size of our study is relatively small, especially since strata of the sporadic and HNPCC groups were analysed separately. Notwithstanding, this is the first study to examine possible differences or similarities in risk factors for sporadic and HNPCC colorectal carcinogenesis.

Selection and recruitment of study subjects and methods of data collection may have affected our results. Indications for endoscopy may form a source of selection bias if they differ for cases and controls and are related to dietary habits. Abdominal pain, diarrhoea, and constipation are frequent indications for endoscopy, but usually not related to having adenomas. Conversely, rectal blood loss may be related to having adenomas, but is usually not related to dietary habits. Therefore, these indications for endoscopy are unlikely to have affected our results. Reasons for non-participation may have been different in the population recruited directly by the gastroenterologist in the hospital (both sporadic and HNPCC subjects), than in those recruited by the Netherlands Foundation for the Detection of Hereditary Tumors (HNPCC subjects only). However, since participation rates were high for both recruitment procedures, this is also thought not to have materially affected the results.

Table 4
Meat preparation and the risk of colorectal adenomas in the sporadic and HNPCC group

	Sporadic populations			HNPCC populations		
	Controls/cases[b]		OR (95% CI)[a]	Controls/cases[b]		OR (95% CI)[a]
Habitual meat preparation						
Temperature of heat source during browning of meat						
Low/medium with every meat type	32	31	1.0	32	24	1.0
Depends on meat type	14	13	0.8 (0.3–2.1)	29	19	0.8 (0.3–1.8)
High with every meat type	17	13	0.9 (0.3–2.3)	17	16	1.0 (0.4–2.7)
Addition of water during browning						
Water added with every meat type	16	9	1.0	11	12	1.0
Depends on meat type	32	32	1.9 (0.7–5.2)	52	28	0.5 (0.2–1.4)
No water added with any meat type	16	16	2.0 (0.6–6.5)	15	20	1.2 (0.4–3.8)
Use of a lid[c]						
No closed lid with any meat type	35	31	1.0	39	39	1.0
Closed lid with some meat types	18	10	0.8 (0.3–2.1)	24	13	0.6 (0.2–1.4)
Closed lid with most meat types	10	16	2.6 (0.9–7.2)	15	8	0.7 (0.2–2.3)
Categories by preferred colour of the meat surface						
Colour meat surface[d]						
Light for all meat types	13	14	1.0	11	8	1.0
Depends on meat type	37	40	1.4 (0.5–3.8)	51	37	1.3 (0.5–3.6)
Dark for all meat types	15	3	0.2 (0.0–1.1)	15	13	1.8 (0.5–6.2)

[a] Models are adjusted for age, gender, energy intake and total meat consumption.
[b] 8–10 Individuals are excluded because of missing data on meat preparation questions.
[c] Categories based on % of meat types prepared with closed lid: 0% of meat types, 1–49% of meat types, 50% or more of meat types.
[d] The 2 photos with (very) light colour of the meat surface were scored as light; the 2 photos with (very) dark colour of the meat surface were scored as dark.

Information bias could occur if cases and controls report their past dietary habits in a different way. At the time of filling out the questionnaires, at home shortly after endoscopy, most sporadic patients were unaware of their diagnosis with respect to adenomas. Moreover, controls in the sporadic group reported having bowel complaints more often than did the cases. All analyses have been adjusted for bowel complaints, but this did not markedly affect the results. HNPCC cases and controls were generally more aware of their risk. However, as HNPCC family members, irrespective of their adenoma history, are generally considered to be relatively health conscious due to the very strong family history of cancer, this is not expected to have led to differences between cases and controls. In the sporadic group, all adenomas were diagnosed during the study period (December 1995–February 1998). Sporadic cases were excluded if they had previous adenomas more than 3 years prior to the adenoma diagnosis included in this study, as their dietary habits may have changed with a long history of repeated endoscopies and colorectal adenomas. In the HNPCC, group, however, adenoma cases were included prospectively (i.e., diagnosis during the study period) as well as retrospectively (i.e., diagnosis before the study period). Additionally, HNPCC adenoma cases were allowed to have a history of adenomas, although the most recent diagnosis of a colorectal adenoma is the one included in this study. Exclusion of HNPCC adenoma cases diagnosed before the study period, as well as exclusion of adenoma cases with a history of previous adenomas, did not materially change the Odds Ratios. There were no marked differences between the sporadic and HNPCC populations in several lifestyle characteristics and energy and macronutrient intakes. Adenoma characteristics related to stage of disease (i.e. multiplicity, dysplasia, villocity) were also not significantly different in sporadic and HNPCC adenomas. Therefore, we do not expect that small differences will have biased the results to a relevant extent.

The meat consumption and preparation questionnaire is designed specifically to estimate meat consumption and meat preparation methods with respect to the formation of heterocyclic amines. It is adapted to Dutch meat preparation methods and uses photos from a very extensive Swedish questionnaire [28]. If linked to a database on contents of specific heterocyclic amines in meat, a questionnaire with this limited number of items and type of questions, could in theory explain a large part of the variation in heterocyclic amine intake [29].

Small and non-significant increases in sporadic adenoma risk were observed for ''not adding water with any meat type'' and for ''closed lid with most meat types''. These results are in line with observations in an experimental setting, where we observed that 'no

addition of water' and 'use of lid to cover pan' were both strongly related to high temperature at the meat surface (unpublished observations). We did not observe a positive association between adenoma risk and preferred colour of the meat surface. Possibly, the colour of the meat surface resulting from habitual preparation in the Netherlands, where there is a preference for "well done" meat, is determined not only by cooking temperature, but also by duration of cooking. Cooking temperature is a far more important determinant of heterocyclic amine formation, and may therefore be better reflected by preparation characteristics such as "not adding any water" and "use of lid to cover pan", instead of the colour of the meat surface.

With respect to the exposure of interest, our hypothesis derives from epidemiological observations in the general population at risk of colorectal cancer, excluding hereditary cancers. However, we questioned whether populations with a hereditary background risk (such as HNPCC families) are subject to similar environmental risk factors. Interestingly, there is some evidence that the molecular pathways leading to cancer involve somatic mutations that are similar in these two groups. The *APC* gene for instance, is known to be involved in both sporadic colorectal cancer and HNPCC colorectal cancer [9]. Abnormalities in the K-*ras* and *TP53* genes, known to be highly frequent in sporadic colorectal cancer, also occur in HNPCC carcinomas [10,11,30–32]. We have previously shown that in this study population there were no major differences in gene involvement of K-*ras* and *TP53* in the sporadic and HNPCC adenomas [12].

Despite similarities regarding the genes involved, our results suggest that there may be relevant differences in the aetiology of sporadic and HNPCC colorectal cancer, i.e. in the early adenoma stage. In studies in rats it has been shown that heterocyclic amines may induce specific mutations in the *APC* gene [33]. It may be postulated that meat consumption and preparation could be related to *APC* gene involvement in sporadic adenomas, whereas in HNPCC different *APC* mutations of an endogenous nature may originate from the mismatch repair phenotype.

In conclusion, in our study of HNPCC family members meat consumption and meat preparation were not associated with an increased risk of adenomas. Although no significant associations between meat consumption and preparation and adenoma risk were observed, some characteristics of habitual meat preparation methods in the Netherlands non-significantly increased the risk of sporadic adenomas and deserve further study.

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