

Nutrition and Cancer, 2008, **60**(2), 131–144

Processed Meat and Colorectal Cancer: A Review of Epidemiologic and Experimental Evidence

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Processed meat intake may be involved in the etiology of colorectal cancer, a major cause of death in affluent countries. The epidemiologic studies published to date conclude that the excess risk in the highest category of processed meat-eaters is comprised between 20 and 50% compared with non-eaters. In addition, the excess risk per gram of intake is clearly higher than that of fresh red meat. Several hypotheses, which are mainly based on studies carried out on red meat, may explain why processed meat intake is linked to cancer risk. Those that have been tested experimentally are (i) that high-fat diets could promote carcinogenesis via insulin resistance or fecal bile acids; (ii) that cooking meat at a high temperature forms carcinogenic heterocyclic amines and polycyclic aromatic hydrocarbons; (iii) that carcinogenic N-nitroso compounds are formed in meat and endogenously; (iv) that heme iron in red meat can promote carcinogenesis because it increases cell proliferation in the mucosa, through lipoperoxidation and/or cytotoxicity of fecal water. Nitrosation might increase the toxicity of heme in cured products. Solving this puzzle is a challenge that would permit to reduce cancer load by changing the processes rather than by banning processed meat.

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Introduction

Colorectal cancer (CRC) is a major cause of cancer death in affluent countries, notably the United States and Western Europe. Diet would strongly influence CRC risk, and changes in foods habits might reduce up to 70% of this cancer burden (1-3). Epidemiologic studies suggest that meat intake is associated with CRC

risk, although the association is not significant in most studies. Published in 1997, the World Cancer Research Fund authoritative expert report states: "evidence shows that red meat probably increases risk and processed meat possibly increases risk of CRC" (2). Since 2000, three meta-analyses showed that total meat intake is not related to risk, but that red meat intake is a significant risk factor. In addition, as reported below, the association of CRC risk with processed red meat may be stronger than that with fresh red meat (4-6). Several hypotheses could explain how processed meat could increase CRC risk, and experimental studies have been carried out accordingly. The major hypotheses that have been tested experimentally are (i) that high-fat or high-protein diets could promote carcinogenesis; (ii) that cooking meat at high temperature forms mutagenic and carcinogenic heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs); (iii) that potentially carcinogenic N-nitroso compounds (NOCs) are formed in food and/or endogenously by nitrosation of amines and amides; (iv) that heme iron in red meat can promote carcinogenesis because it increases cell proliferation in the colonic mucosa, through lipoperoxidation and/or cytotoxicity of fecal water. Few experiments have been directly carried out on processed meats but the studies undertaken on red meats make it possible to propose the hypotheses cited above. There are no clearly demonstrated biologic mechanisms that could explain the risk difference between processed and unprocessed meat.

The aims of the present paper are (i) to describe briefly the processed meat products (ii) to review the epidemiologic evidence that processed meat increases CRC risk, (iii) to review the experimental studies on the mechanisms explaining the effect of processed meat on colorectal carcinogenesis.

Processed Meat

Processed meat is made mostly from pork or beef meat that are preserved by methods other than freezing, and that undergo a treatment to improve the quality of cuts of carcasses, to increase preservation, and to change flavor. There is a huge variety of processed meat products and it is not easy to sort them by categories, but parameters involved in the making of these foods are curing (adding salt and other additives), drying, smoking, cooking and packaging. Processed meat includes bacon, ham (raw, smoked or cooked), heated sausages like hot-dogs (frankfurters), raw sausages (like salami), bologna, blood sausage (UK: black pudding), liver pâté (or liverwurst) and other pâtés and spread meat, luncheon meat and other cold cuts, canned meat, and corned beef (7, 8). This list is not comprehensive, and many other specific products are made all over the world, using traditional recipes. Curing and smoking, two specific processes for meat, are described below as they might generate potential hazards.

= **Curing** is the addition of a combination of salt, sugar and either nitrate or nitrite: salt improves the taste of meat and preserves it by stopping bacterial growth, because it diffuses inside the muscle and reduces the water activity. Nitrite inhibits the germination of *Clostridium botulinum* spores, and gives the meat the desirable cured color by combining with heme iron. Nitrosylmyoglobin is responsible for the red color of raw cured meat. Cooking denatures globin which detaches from the heme, yielding a pink mononitrosylheme complex, the color of cooked cured meat (9, 10). When saltpeter/nitrate is used, a previous step is needed so that bacteria reduce nitrate to nitrite. In many countries, the maximum permitted concentration of nitrite in processed meat is 200 ppm, and it is 150 ppm in the European Union. Curing can be done with dry salt, in a brine tank, or by injection:

- Dry salting is the old way of meat curing. Cuts of meat are placed on heaps of salt and rubbed with salt or with a mix of salt, sugar and saltpeter (11). This treatment is simple, but long, and its efficacy depends on the diffusion of salt into the meat.

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- Tank curing is faster than dry salting: meat pieces are placed in brine, water saturated with salt that may also contain sugar and nitrite.
- Methods have been developed to accelerate the rate of diffusion of curing agents into meat either by the use of the arterial system by needle injections, or with multi-needle system. Moreover, new additives have been used in brine to improve the color formation and stability with reducing agents like sodium ascorbate or erythorbate.

= **Smoking** is the process of exposing meat to the smoke from incomplete wood pyrolysis. This gives meat a brown color, changes its flavor and helps its preservation because smoke contains phenols, aldehydes, acetic acid and other carboxylic acids. Wood pyrolysis may generate carcinogenic polycyclic aromatic hydrocarbon (PAH), and the process is hard to control. A more controlled process is obtained by immersing meat pieces into a "smoke solution", which gives smoke flavor without PAH contamination, and improves meat preservation because it contains acetic acid.

Among the many existing processed meat products, we chose to describe ham and sausages that contribute most to the overall processed meat intake (12). Ham is obtained by curing the upper quarter (thigh and sirloin) of a pig, and may be boiled (Jambon de Paris), dried (country ham), and/or smoked. Sausages are prepared with chopped meat (pork usually, or a mix of pork and beef), lard, salt, and other additives (e.g., wine, saltpeter, garlic, herbs, spices). This preparation is usually packed in a casing (historically the intestines of the animal, though now often collagenic, cellulosic or polymeric). Sausages may be dried (salami-type), cooked (hot-dog type), and/or smoked. Blood sausage (UK black pudding) is prepared with blood (usually from pork), lard or suet, and a plant-based filling (bread, barley, onions), in three

equal parts, with salt and spice. This preparation is packed in a pork bowel, and cooked until it becomes thick.

Processed meat intake makes one half to one fifth of total red meat intake. For instance, in 1999 French adults ate 38 and 63 g/d processed and fresh red meat, respectively (13). In Europe, the intake of processed meat was 27 g/d [11-48] in women (median and range of 23 EPIC centers from ten European countries), and 48 g/d [19-88] in men (12). Fresh red meat intake was 36 g [25-52] in women, and 60 g/d [40-120] in European men (7). In the American CPSII Nutrition Cohort (median age 63 years) the median intake of processed and fresh red meat was estimated as 10 and 40 g/d, respectively (14). In a Bethesda case-control study (median age 58 years) the mean intake of processed and red meat was 12 and 36 g/d (15, 16). These values may be underestimated, since they are based on food-frequency questionnaire data, and because subjects were older than the general population. Indeed, Norat et al. estimated that red meat intake in North America is 72 g/d per caput (5).

Epidemiologic Studies

International ecological studies show that countries where people eat more red meat are also countries where the risk of CRC is high (17). Analytical studies suggest that this association is also seen at the individual level, but the link is significant in only one study out of three (18). Three meta-analyses have been published since 2000, and their quantitative risk estimate for fresh red meat and processed meat intake are summarized below and in Table 1.

Sandhu et al. (2001) made a meta-analysis gathering 13 cohort studies, selected from 17 studies, according to pre-established quality criteria (4). All cohorts' studies with relative risks between meat/processed meat intake and colon/colorectal cancer incidence or mortality were included up to 1999. Prospective studies that did not report the level of exposure (red meat/processed meat consumption) were excluded. Norat et al. (2002) study derives 18 case-control and 6 cohort studies, selected from 48 (5). All studies published up to 1999, and

providing association between total meat, red meat or processed meat intake and colon, rectal and colorectal incidence or mortality, were included. Sandhu's and Norat's meta-analyses were not independent, since eleven studies were common to both articles. Last, Larsson et al. published in 2006 a meta-analysis of 18 prospective studies, selected from 23, gathering a total of more than one million subjects. Norat's and Larsson's studies were quite independent, since only 15% of Norat's subjects were counted again in Larsson's study (6).

These three meta-analyses take all previous studies into account, and bring global and consistent conclusions on the effect of different types of meat: total meat, red meat, processed meat. Briefly:

- Total intake of meat (including white and red meat from all sources) is not associated with CRC risk in Norat's and in Larsson's analyses. Sandhu's study shows a significant moderate risk associated with total meat intake, but the authors did not include white meat (poultry) in total meat.

- A high intake of red meat (usually including beef, veal, lamb, mutton, pork, and offal) is associated with a moderate and significant increased risk of CRC in the three studies:

- = In Sandhu's study (4), the average relative risk (RR) of CRC for a 100 g portion of red meat is 1.17. The 95% confidence interval (CI) is 1.05-1.31. Processed meat was not included in red meat, we think, in this study (4).

- = In Norat's study (5), CRC RR = 1.35 (CI: 1.21-1.51) for the highest quartile of consumption of red meat (including processed meat). A minor difference is observed between results from case-control and cohort studies (RR=1.36 and 1.27 respectively). The intake of 120 g/d of fresh (unprocessed) red meat is associated with a significant risk, but of lower magnitude than when processed meat is included (+ 19% compared with + 35%) (5).

- = In Larsson's study (6), CRC RR = 1.28 (CI: 1.15-1.42) for the highest category of consumption of red meat (including processed meat). Fresh red meat intake (unprocessed meat) was reported in nine

studies out of fifteen, and the associated RR was 1.22, a significant value. The risk excess associated with intake of 120 g/d of red meat was + 28%. Larsson's article does not report the quantitative effect of fresh red meat, and no precision is given on the categories (6).

- Processed meat intake (usually including sausages, meats burgers, ham, bacon, salami, nitrite-treated meat and meat products) is associated with CRC risk in all reports: Global RR are 1.49 (CI: 1.22-1.81), 1.31 (CI: 1.13-1.51) and 1.20 (CI: 1.11-1.31) in the three meta-analyses (4-6). In Norat's analysis, a minor difference is observed between results from case-control and cohort studies (RR=1.29 and 1.39 respectively).

Thus the estimated excess risk associated with fresh red meat intake was 17%, 19% and 22%, and the risk associated with processed meat was 49%, 31% and 20%, in the three reviews, respectively. The estimates of risk for fresh red meat are within a narrow range, but estimates of risk for processed meat are more dispersed. However, all RRs are significant, and none is larger than 1.5, which shows the consistency of the meta-analyses. As shown in Table 1, doses-response meta-analyses suggest that one gram of processed meat is eleven-times, six-times or twice more "promoting" than one gram of fresh red meat in the three meta-analyses, respectively (4-6). It is not easy to explain why the processed/fresh meat ratio is higher in Sandhu's study than in Larsson's study. However, the three studies indicate that processed meat intake is associated with a higher CRC risk than the intake of other types of meat.

Four cohort study articles dealing with processed meat intake and CRC have been published after Larsson's 2006 review (one new cohort, and three re-analyses, Table 2), and seven case-control studies shown in Table 3 were published after Norat's 2002 review. Let us examine below if they strengthen or weaken the above-reported meta-analyses results.

= A cohort of 30,000 men and women in Japan was studied by Oba et al. (2006), with 231 CRC cases. Processed meats were ham, sausage, bacon, and yakibuta (Chinese roasted pork). In men, there was a positive association between CRC and the highest tertile of processed meat consumption (RR=1.98, CI: 1.24-3.16). No

association was seen in women (RR=0.85, CI: 0.5-1.43) (19). Three other articles made use of already published cohort studies, but they analyzed prospective data by dietary patterns, instead of type of foods. Fung et al. (2003) used data from the Nurses' Health Study (20). The highest quintile of women eating a "western pattern", defined by a high intake of red and processed meats, sweets and desserts, French fries, and refined grains, had a marginally significant increase in colon cancer risk, consistent with meta-analyses result (RR= 1.46, CI: 0.97-2.19). No association was found with rectal cancer (20). Dixon et al. (2004) analyzed three prospective studies: the Alpha-Tocopherol Beta-Carotene Study (ATBC), the Netherlands Cohort (NLC), and the Swedish Mammography Cohort (SMC) (21). Exploratory factor analysis identified a dietary pattern that includes processed meat in the three cohorts: the Processed meat, Pork, and Potatoes pattern. This pattern was associated with an increased risk of colon cancer in the SMC women (RR=1.62, CI: 1.12-2.34), and of rectal cancer in the ATBC men (RR=2.21, CI: 1.07-4.57), but not in the NLC study (RRs=0.9) (21). Kesse et al. (2006) studied food patterns in a French cohort of women, already reported in the EPIC study. The "Western" diet pattern included: processed meat, potatoes, pizzas and pies, sandwiches, sweets, cakes, cheese, cereal products, eggs, and butter. The three other diets were: "Healthy" diet (vegetables, fruit, yogurt, sea products, and olive oil, "Drinker" diet (sandwiches, snacks, processed meat, and alcoholic beverages) and "Meat eaters" diet (meat, poultry, and margarine). "The" Western pattern increased adenoma risk, but not CRC risk (RR= 1.39, CI: 1.00-1.94 and RR = 1.09, CI: 0.60-2.00 respectively). "The" Drinker and the Meat eaters diets increased the adenoma risk and the CRC risk (see RRs on Table 3) (22). To sum up these recent prospective studies, they bring some support to the conclusions of Larsson's meta-analysis that processed meat intake is associated with increased risk, and the RR is in the range 1.5-2. However, the link was not found in all sub-groups (male/female, colon/rectum), and the risk associated with dietary patterns cannot be attributed to processed meat alone.

= Seven case-control studies dealing with processed meat have been published after Norat's

meta-analysis. All studies report OR above 1.15, but only three studies out of six found a significant association between processed meat intake and CRC risk. In Shanghai, China, Chiu et al. (2003) found that a high intake of preserved foods (whether animal or plant source) was associated with an increased risk of colon cancer (OR= 2.0, CI: 1.5-2.9 in men, and OR=2.7, CI: 1.9-3.8 in women). Preserved vegetables was more strongly associated with cancer risk than preserved animal foods (23). In the U.S.A., Chiu and Gapstur (2004) investigated the effect of dietary changes during adult life. They showed that risk was higher for people who did not reduce their consumption of red meat and processed meat after the age of 30 years, and risk was particularly high for pork chops/ham steaks eaters (OR= 3.7, CI: 1.6-8.7) (24). In Canada, Nkondjock et al. established dietary patterns, as reported above for cohorts. The “pork and processed meat” pattern, characterized by a high consumption of processed meat, pork, and white bread, increased colon cancer risk nearly significantly (RR=1.6, CI: 0.9-2.8) (25). In Utah and Northern California, Murtaugh et al. (2004) found no association between processed meat intake and the risk of rectal cancer (RR=1.2, CI: 0.85-1.7) (26). In Japan, Kimura et al. found that processed meat intake (and red meat intake) was not related to CRC risk (OR=1.15, CI: 0.83-1.60) (27). A Maryland case-control study of colorectal adenoma found a two-fold increased risk in the highest, compared to the lowest, quartile of processed meat intake (95% CI = 1.0-4.0). This OR was mostly explained by nitrate/nitrite intake, and marginally attenuated by MeIQx intake (a heterocyclic amine formed by cooking). In addition, ham steak/pork chops, hot dogs/other sausages, and liverwurst intake each were associated with a two-fold risk of adenoma, while bacon, breakfast sausages, ham, bologna, salami, and other luncheon meats intake were not associated with the risk (16). Lastly, In Canada, Hu et al. (28) found that consumption of processed meat increase risk of both proximal and distal colon cancer in men and women (all four OR were between 1.4 and 1.6, all CI:1.0-2.0, 2.2 or 2.4). Bacon intake was particularly associated with risk of colon cancer (proximal and distal) in women.

The estimation of cancer risk associated with meat may be influenced by other dietary factors,

as shown clearly in the "dietary pattern" studies cited above (20-22). In those studies, the intake of processed meat was associated with intake of French fries (or potatoes), sweets, cakes, desserts, snacks and alcoholic beverages: These high glycemic index diets, and alcohol intake, may be risk factors for colorectal cancer. In addition, high-meat diets have been negatively associated with food groups rich in antioxidants and fiber, components which have been associated with a reduced risk of colorectal cancer (4). Thus, the effect of processed meat consumption on the risk of colorectal cancer may be confounded by other foods, as discussed further in the "Indirect mechanisms" section below. However, red meat intake is more consistently associated with risk than any other dietary factor, except the total energy intake (3, 29).

In summary, the results of these meta-analyses support the hypothesis that high consumption of red and processed meat may increase the risk of CRC. The few studies published after the meta-analyses also support the evidence, although individual studies are seldom significant. In addition, the risk associated with consumption of one gram of processed meat was two to ten times higher than the risk associated with one gram of fresh red meat. It is thus likely that processed meat contains some components that are more potent than fresh red meat components.

Mechanisms of Processed Meat Promotion: Experimental Data in Rodents and Volunteers

Several hypotheses may explain why processed meat intake is linked to CRC risk. Processed meats often differ from red meat by three major points: (i) they often contain more fat than red meat; (ii) they contain specific additives, notably salt and sodium nitrite; (iii) their long-time storage yields cholesterol oxidation products. Like red meat, processed meat is rich in fat, protein and heme iron, which can promote carcinogenesis, or yield promoters *in vivo*. Processing and cooking can generate heterocyclic amines (HCAs), polycyclic aromatic hydrocarbon (PAHs), and N-nitroso

compounds (NOCs). Specific HCAs, PAHs and NOCs are mutagens and animal carcinogens. In addition, people eating a large amount of processed meat may lack protective phytochemicals and/or be at increased risk due to sedentary life-style, obesity and/or insulin resistance. These hypotheses are considered sequentially below.

1- Fat

Epidemiologic studies and laboratory animal models suggest that a high intake of dietary fat promotes CRC. High fat intake favors the secretion of bile acids (BA) into the duodenum, and activates bacterial 7-alpha-dehydroxylase that makes secondary BA. These BA, deoxycholic and lithocholic acids, promote colon carcinogenesis in several animal models, and are elevated in stools from populations at risk for cancer (30). A high fat diet also leads to free fatty acids in the colonic lumen. They may damage the colonic epithelium and increase proliferation, an effect blocked by dietary calcium (31). The hypothesis that fat or BA promotes colorectal carcinogenesis have been tested in several studies briefly reported below.

Four studies gave direct evidence that a high-fat diet can increase carcinogen-induced tumor in the colon of rats. Reddy et al. (1976) showed that protein and fat from meat increase the incidence of colon tumors in 1,2-dimethylhydrazine (DMH) injected F344 rats (32). A 30% beef tallow diet given after carcinogen injections increased the tumor yield, compared with the 5% fat diet fed controls. In contrast, the high fat diet had no effect when given simultaneously with the carcinogen (33). Last, Pence et al. (1995) showed that a 20% fat diet significantly increases the number of adenoma in the colon of DMH-initiated rats, whatever the protein and the fat sources (meat, casein, corn oil or beef tallow) (34).

In contrast, several studies showed no effect of saturated fat on colorectal carcinogenesis. Nauss et al. (1983) found that a 24% beef tallow diet did not enhance CRC in Sprague-Dawley (SD) rats (compared with 5% fat diet controls) (35). Nutter et al. (1983) failed to show a promoting effect by beef tallow in BALB/c mice, though corn oil was promoting

(36). Clinton et al. (1992) did not find any promoting effect of diets with 24% or 48% calories from corn oil (compared to 12%) in SD rats (37). No difference in epithelial cell proliferation rate was observed in rats fed diet with 10%, 25% or 40% of the energy derived from fat (38). Beef tallow diet (14%) reduces the number of aberrant crypt foci and increases apoptosis in the colon of DMH-initiated rats, compared with soybean oil diet controls (39). A meta-analysis of rat studies can explain these puzzling discrepancies: saturated and n-6 polyunsaturated fats are promoters in F344 rats, but SD rats resist to high-fat diet promotion (40).

Bile acids have been known to be tumor promoters for many years, and the addition of cholic acid to rodents diet enhances colonic epithelial cell proliferation, and increases the number of tumors in animals exposed to carcinogens (reviewed in (30)). Blood BA also correlates with tumor incidence in F344 rats (41). However, the hypothesis that saturated fat intake enhances fecal BA excretion was not supported by several studies in rats and in volunteers. Compared with soybean oil diet, beef tallow reduces fecal BA in DMH-induced rats (39). Another Gallaher's study shows that low-fiber diets rich in beef tallow (20%) decreases BA concentration in the colon and do not increase colon carcinogenesis in rats (42). In human volunteers too, a high-fat diet (53% compared with 14% energy) does not change BA concentration in stools, although it increases colonic nuclear aberration (43). Thus, although populations consuming higher amounts of fat have higher levels of BA, the difference do not show up clearly in case-control studies and in experimental interventions (30).

Fat intake has been suggested in the past as a major factor that could explain the link between CRC and meat intake. However, experimental studies reported above are not consistent, and recent epidemiologic studies failed to confirm previous reports: Red and processed meat intake, but not fat intake, remains a major risk factor for CRC (3, 29). High-fat diets are high-calorie diets, and the balance between energy intake and physical activity is still considered a major risk factor.

Kumar et al. attempted to disentangle the effect of fat and calories, with a pair-feeding design (44). Each rat was given each day a weighed meal, lighter than the ad libitum intake, so that control and treated rats got the same amount of calories. In rats fed ad libitum, a high fat diet promotes colon tumor compared to a low fat diet (85 vs. 56% incidence, $p < 0.05$). In 20% calorie-restricted pair-fed rats, the high fat diet also increased tumor yield compared with low fat diet, but the effect was no longer significant (56 vs. 41% incidence). This study suggests that both fat and calories are promoting factors (44). It is thus possible that fatty processed meat increases CRC risk because it brings too much energy to the customer as reported in the last section 6-iv.

2- Heterocyclic Amines (HCAs) and Polycyclic Aromatic Hydrocarbons (PAHs)

HCAs and PAHs are formed during the cooking of meat. HCAs are formed by pyrolysis of creatine with specific amino-acids. Since a high temperature is needed, only fried, broiled, or barbecued meat contains significant amounts of HCAs (45). Various HCAs are formed according to the type of meat, the heating temperature, and the chemical environment (e.g., water, oil, onion). Processed meat from pork does not contain a particularly large amount of HCAs compared with pan-fried beef and chicken (46). Most abundant HCAs in meat are 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4,9-trimethylimidazo[4,5-f]quinoxaline (DiMeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) (45).

In contrast with HCAs, PAHs are produced from the incomplete combustion of organic compounds. Many tested PAHs, like benzo[a]pyrene (BaP), are mutagens and animal carcinogens. The main sources of PAHs for humans are cooked and smoked meat and fish, notably barbecued meat, and tobacco smoke (47). Furthermore, nitrosation of HCAs such as MeIQx or IQ has been proposed as a mechanism by which well-done red meat consumption and inflammation can initiate colon cancer under inflammatory conditions, such as colitis. This mechanism is potentiated by heme (48).

Epidemiology suggests that cooking methods and doneness of meat are related to CRC risk, higher temperature leading to higher risk. A 1991 Swedish case-control study showed that frequent consumption of fried meat with a heavily browned surface led to 3-fold increase in CRC risk (49). Since 1991, some twenty similar analytical studies have been published. Briefly, most studies, but not all, confirm the Swedish findings: the intake of grilled, fried, barbecued and/or well-done red meat is more related to CRC risk than the intake of total red meat (OR comprised between 1.3 and 4). Some studies specifically addressed the effect of metabolic phenotype on the response to well-done meat intake (50). The association of PAH intake and adenoma risk was recently studied in two case-control studies by the same team. Barbecued meat and PAH intake, but not broiled meat or HCA intake, was strongly related to the risk of bearing an adenoma (51, 52).

Experimental studies of HCAs started with Sugimura's discovery that cooked fish extract contains potent mutagens. HCAs were shown later to be complete carcinogens, and to induce colon, mammary and prostate tumors in rodents and in monkeys (53). However, carcinogenic doses in rodents are 1000-100,000 times higher than those that are found in cooked meat (54, 55). Only one publication reports the effect of well cooked beef diet, with a high HCA content (measured by HPLC). This cooked meat promotes CRC in DMH-initiated rats in a low-fat diet context, but surprisingly not in high-fat diet context (56). Based on ancient carcinogenicity studies, PAHs and BaP were supposed not to induce CRC in animals, but Tudek et al (1989) showed that BaP can induce ACF in the colon of mice (not of rats) (57). O'Neill et al. (1991) reported that BaP gavages induce colonic nuclear aberrations in mice fed a human diet (58). In humans, BaP forms DNA adducts, evidenced by HPLC with fluorescence detection, in colonic mucosa of humans (59).

To sum up, traces of HCAs and PAHs are present the daily diet of meat-eaters, but not specifically in processed meat. They are proven carcinogens, and may lead to colonic tumors. We however believe that HCAs are not major determinant of CRC in humans, based on the following facts:

- Chicken meat is a major contributor of HCA intake, but its consumption is not associated with CRC risk in epidemiologic studies (5, 6, 8)
- The dose of HCAs that leads to CRC in rodents and in monkeys is 1000 to 100,000 times higher than human exposure through cooked meat (54, 55).
- Colon cancer risk in humans is more related with cooking methods than with HCA intake (60, 61).

However, HCA metabolism is different in rats and in humans, and specific human genotypes (e.g., rapid NAT2 and CYP1A2) are at a high risk for CRC. Recent case-control studies suggest that PAHs may be better candidates than HCAs, to explain that overcooked meat is a CRC risk factor, but data on PAHs are insufficient to conclude.

3- Nitrite and N-Nitroso Compounds (NOCs)

NOCs, which are alkylating agents that can react with DNA, are produced by the reaction of nitrite and nitrogen oxides with secondary amines and N-alkylamides. Many NOCs, including nitrosamines and nitrosamides, are carcinogenic in laboratory animals. Humans can be exposed to NOCs by exogenous routes from certain processed meats (e.g., grilled bacon), smoked fish, cheeses or beers (62). In acidic conditions such as those found in processing procedures of meat, dinitrogen-, tri-, and tetraoxides can form and these are nitrosating agents. In a large-scale Finland cohort, N-nitrosodimethylamine intake from smoked and salted fish, and cured meat, was associated with CRC risk (RR=2.12, CI:1.04-4.33), but nitrite intake was not related to risk (63). Humans can also be exposed to NOCs by endogenous routes, and a high-red meat diet leads to the endogenous synthesis of NOCs in volunteers (64). Decarboxylation of amino acids by gut bacteria yields amines and amides that can be N-nitrosated in the large bowel (65). Heme from meat strikingly increases NOC formation (66), even in the absence of colonic flora in the upper gastrointestinal tract (67). Ascorbic acid is often added to processed meat, as an antioxidant additive. Since it prevents nitrosation, it may reduce the formation of NOCs in foods and in the digestive tract (68).

Animal studies showed that processed meat intake leads to fecal excretion of NOCs, but without any evidence of initiation or promotion of ACF. Parnaud et al. showed in three independent studies that grilled bacon-fed rats excrete 10 to 20 times more NOCs in feces than do controls (9-22 vs 0.5-1.4 nmol NOC/g feces), a difference mostly due to NOC intake. In contrast with human studies, rats fed a diet based on pork or beef meat had less fecal NOCs than controls. However, in bacon-fed rats, these NOCs did not initiate ACF at 45 days, nor did they promote ACF at 100 days after an AOM-injection (69). Mirvish et al. showed that hot dogs contain 10 times more NOCs than fresh red meat. Both meats also contained one thousand times more NOC precursors than NOCs (70). Mice given a hot-dog diet (18%) had 4-5 times, and beef-meat fed mice 2-3 times, more NOCs in feces than no-meat fed controls (71). Mirvish and coll. are still working to find the precise nature of NOC precursors in hot dogs, a major one being 1-deoxy-N-1-glucosyl glycine, and if these NOCs are mutagenic on bacteria (72).

Human studies showed that dietary beef meat, but not poultry, strikingly increases NOC excretion in feces. Bingham et al. evidenced a three-fold increase in fecal NOCs in volunteers who consumed diets high fresh red meat diet (600 g/day, compared with 60 g/d in controls) (64). They also showed the formation of DNA adduct O6-carboxymethyl guanine in colonic exfoliated cells or meat-fed volunteers (73). This NOC-specific alkylating DNA adducts suggest that increased endogenous production of NOCs may be relevant to the etiology of CRC. The same team showed that NOC production depends on the quantity of dietary red meat, and that the amount of NOCs increases 30-times during the transit between mouth and feces (74). White meat intake does not yield NOCs, and fibers or vegetables intake does not counteract red meat production of NOCs in volunteers (75). Cross et al. showed that dietary heme, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat (66). Heme from fresh red meat or from processed meat (240 g/d each) given to ileostomists led to a 4-fold or 6.5-fold increase in the amount of endogenous NOCs excreted in the ileostomy output, respectively. Heme thus facilitates the formation of NOCs in the absence

of colonic flora in the upper gastrointestinal tract. From *in vitro* evidence, authors suggest that nitrosyl-hemoglobin is the major nitrosating agent in the digestive tract (67).

In conclusion, according to the International Agency for Research on Cancer, ingested nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A (IARC Monogr Eval Carcinog Risks Hum. 2007, 94: in the press). NOCs are present in some processed meat, and are formed endogenously after red and processed meat consumption. Heme is a major determinant of NOC formation, and nitrite also contributes to NOC yield. Although many tested NOCs induce cancer in rodents, and NOC-adducts are found on volunteers' colonic DNA, it is not yet clear whether red and processed meat-induced NOCs are colon carcinogens.

4- Heme

Heme (UK, haem) consists of an iron atom contained in the center of a large heterocyclic organic ring called a porphyrin. Heme is included in muscles myoglobin, in red blood cells hemoglobin, and in cytochromes. Blood sausage and liver paté are particularly rich in heme, followed by dark red meat products, but chicken meat contains little heme. Two prospective cohort studies recently found that a high intake of heme iron was associated with a higher risk of CRC. The relative risk was 2.18 (CI: 1.24-3.86) in the Iowa Women's Health Study cohort (76), and 1.31 (CI: 0.98-1.75) in the Swedish Mammography Cohort (77). In this cohort, a significant RR of 1.26 (CI: 1.02-1.55) was associated with the consumption of two servings of blood sausage per month (77). Three mechanisms may explain heme promotion of carcinogenesis: (i) heme is metabolized in the gut into a cytotoxic and promoting factor (78); (ii) heme induces peroxydation of fat in foods and in the gut, and the lipoperoxides would promote CRC (79); (iii) heme catalyzes the endogenous N-nitrosation, which increases the formation of NOCs, as reported above (66), and the activation of HCAs (48).

(i) In rats fed a low-calcium diet, Sesink *et al.* (78) showed that dietary hemin increases epithelial proliferation in the colonic mucosa,

and induces cytotoxicity of fecal water. Hemin, a free heme ring stabilized by a chlorine atom, was fed to the rats for 14 days. Hyperproliferation may be considered as a compensation for the cytotoxicity (78). This effect was shown repeatedly and dietary calcium and chlorophyll could block the hemin effect. Hemin-fed rats excrete much less host DNA in feces than controls, which suggests that hemin decreased cell differentiation and exfoliation of colonocytes in the gut lumen (80). However, the above cited studies have all been conducted with hemin, not with food heme, and the speculated heme-based cytotoxic factor has not yet been identified.

(ii) In azoxymethane (AOM)-initiated rats given a low-calcium diet, dietary hemin and hemoglobin promote dose-dependently the growth of colon AFC (81). Meat-based diets also promote ACF and mucin depleted foci (MDF) in rats: MDF promotion by the high-heme blood sausage diet was greater than that by the medium-heme beef diet, but low-heme chicken diet did not promote MDF (82). The high-heme meat diets also increases the formation of lipoperoxides as malondialdehyde in the gut lumen (82), and the excretion of a lipoperoxydation biomarker, 1,4-dihydroxynonane mercapturic acid, in the urine of rats. The same marker is found in the urine of blood sausage-fed volunteers (83). Also, a DNA adduct derived from lipid peroxidation, malondialdehyde-deoxyguanosine, is found in higher levels in the cells shedded in fecal stream from adenoma patients than from controls (84). *In vitro*, hemin and hemoglobin are toxic and genotoxic in colonic cell line HT29 and in primary culture of human colonocytes. Mechanisms would imply the uptake of iron by cells, followed by free radicals oxidative stress (85).

The hypothesis that heme explains the link between meat intake and CRC risk is consistent with epidemiologic studies: red meat, not white meat, intake is related to the risk. However, most processed meat products are of porcine origin and contain less heme iron than beef meat. What would explain that processed meat is associated with higher risk than fresh meat? We suggest that heme form in food makes a difference. As reported above, in raw cured meat the myoglobin heme is nitrosylated. Further

cooking releases nitrosylheme from myoglobin (9, 10). We speculate that, like hemin, free nitrosylheme could be more toxic than fresh meat myoglobin. Indeed, it shows weak mutagenic activity in the Ames test (86), but its promoting effect remains to be tested in vivo.

5- Unlikely Hypotheses: Protein, Salt, Cholesterol

5.1- Proteins

The evidence of CRC promotion is much weaker for high-protein diets than for high-fat diets (87), and epidemiology studies do not suggest that protein intake is a risk factor. However several mechanisms might explain CRC promotion by high protein diets. A high protein diet, or digestion-resistant proteins, leads to more protein entering the colon and being fermented by the gut microflora (65). Protein fermentation products, like ammonia, phenols and p-cresol, show some promoting properties, possibly because of their toxicity to the mucosa. They disturb cellular metabolism and DNA synthesis, reduce cell life span and enhance cell turn-over (88). One study indeed highlights the promoter effect of ammonia in rodents but it is difficult to conclude definitively in humans due to difficulties in exposure assessment (89). In addition methionine, an amino-acid abundant in meat products, and polyamines, deriving from amino-acids, can directly promote experimental carcinogenesis (90). Few studies addressed the effect of the level of dietary proteins on colon carcinogenesis. High-beef protein and high soybean protein diets significantly increase the incidence of DMH-induced tumors in F344 rats compared with medium-protein control diets (32). In contrast, no difference in aberrant crypt foci (ACF, preneoplastic lesions) yield was seen between rats fed diets containing 8, 16 or 32% barbecued kangaroo meat after azoxymethane injections (91). Eleven studies have tested the hypothesis that meat proteins can promote carcinogenesis in rodents, compared with milk or soybean proteins. Results from these studies do not show a specific tumor promotion by meat. In contrast, as reviewed in two previous articles, the incidence of tumors was lower in meat fed rodents than in casein or soybean fed controls (82, 92).

The level and the nature of dietary proteins do not seem to be major determinants of carcinogenesis, and proteins from meat do not promote experimental carcinogenesis. In contrast, proteins that are slowly or not digested can promote carcinogenesis, as shown with overcooked casein and with potato proteins (88, 93). We have no evidence that processed meat products contain such resistant proteins, but this could be studied in the future.

5.2- Cholesterol Oxidation Products

Long-time storage, fermentation, and/or frying of fatty meat products in the presence of oxygen yield cholesterol oxidation products, a phenomenon inhibited by nitrite addition (94). Dietary cholesterol is not related to CRC risk, and high blood cholesterol is associated with decreased CRC risk. Also, a cholesterol enriched diet decreases the formation of ACF in AOM-initiated mice (95). In contrast, the inclusion of oxidized cholesterol in diet increases the formation of AOM-induced ACF in mice (96). However, although plasma 7 β -hydroxycholesterol has been associated with lung cancer risk in a case-control study cited by (12), few studies were done on the hypothesis that oxysterols might induce or promote CRC, and the evidence is weak. Since most processed meats contain nitrite that inhibits cholesterol oxidation, it is unlikely that oxysterols are the cause of processed meat effect on CRC.

5.3- Salt

Processed meat contains much more salt than red meat, with NaCl concentrations ranging from 1 to 10%. Salty diets and salted foods have consistently been related to stomach cancers, particularly in Japan (97), but no link has been published between salt intake and CRC. In contrast, in rats, a NaCl enriched-diet reduces the number of ACF in AOM-initiated rats (98, 99), likely because water intake is more than doubled in salty diet-fed rats.

6- Indirect Mechanisms: Less Vegetables, More Calories

Individuals who eat more processed meat than average often tend (i) to eat less fruits and vegetables, (ii) to drink more alcoholic beverages, (iii) to smoke more tobacco and (iv) to eat more calories, more fat and be more obese and less active, than those who do not eat processed meat (20-22, 25).

- (i) There is limited evidence for a CRC-preventive effect of the consumption of fruits and vegetables (2, 100), although large-scale intervention studies did not point to a protective effect (3, 101, 102).
- (ii) Alcohol intake is associated with a small increase in risk of colorectal cancer (2).
- (iii) Cigarette smoking is a putative environmental risk factor for colon cancer. In Giovannucci's review (101), the average relative risk of colorectal adenoma is three-fold higher for people who smoked for more than 30 years and with high intensity (20-40 cigarettes/day). PAHs and HCAs are formed when tobacco is burning, and swallowed by the smoker.
- (iv) High-fat diets are high-calorie diets, and excess caloric intake is consistently reported as a major CRC risk factor (1-3, 29). In the large-scale EPIC cohort, abdominal obesity (waist-to-hip ratio) is a risk factor (103), but physical activity reduces the risk (104). In rodents, caloric reduction consistently reduces cancer yield (105). The mechanistic link could be that excess calories induces insulin resistance and high blood glucose, free fatty acids, insulin, and IGF1 which promote tumor growth as speculated first in 1994 (106). The high levels of circulating nutrients and growth factors result in increased proliferation, less apoptosis, activated PPARs, more oxidative stress and chronic inflammation (107, 108). It is thus possible that fatty processed meat increases CRC risk because it provides too much energy to the sedentary customer.

General Conclusion

The fact that processed meat intake increases colorectal cancer risk seems established from the published meta-analyses of epidemiologic studies. The evidence is weak, however, since the RRs were all less than 2, and observational studies never fully avoid biases and confounders. The excess risk in the highest category of processed meat-eaters is comprised between 20 and 50% compared with non-eaters, which is modest compared with established risk factors like cigarette smoking for lung cancer (RR=20). However, the excess risk per gram of intake is clearly higher than that of fresh red meat.

Several hypotheses may explain the association of processed meat intake with CRC risk. From data reviewed above, the authors propose that the most likely explanations for the excess risk in processed meat eaters are (i) heme-induced promoters and (ii) carcinogenic N-nitroso-compounds. These toxic compounds are not specific to processed meat, but it is likely that nitrite curing enhances the toxicity: (i) nitrite binds to the heme iron, and the nitrosylheme could yield more toxic lipoperoxides and/or cytotoxic agents than native myoglobin-bound heme; (ii) nitrite curing leads to increased levels of N-nitrosated compounds in food and in the gut: Processed meat eaters are thus exposed to larger NOC levels than fresh meat eaters.

Colorectal cancer is the first cause of cancer death among non-smokers in affluent countries, and the five-year survival (approx. 60%) improves too slowly with the advances in the treatment of the disease. CRC prevention is thus a major goal for public health. Today, prevention is mostly based on dietary recommendations, notably the advice to reduce or to avoid processed meat consumption (2). We think that the prevention strategy might be improved if the mechanisms of cancer promotion were better understood. We guess that non-toxic processed meat could be produced, either by removing the potential toxic agent (e.g., removing nitrite to reduce NOC formation), or by adding a specific inhibitor, e.g., calcium to block heme in the digestive tract (Pierre et al, 2007, *Brit.J.Nutr.*, accepted manuscript). This would permit the reduction of CRC load, without putting an end to the production and consumption of traditional, nutritional and enjoyable foods.

Acknowledgements

The authors thank the help of Sidney Mirvish and of Jean-Luc Vandevre who carefully read the manuscript and proposed many changes to improve the text.

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Table 1 – Excess risk of CRC associated with the intake of fresh red meat and of processed meat in three dose-response meta-analyses of analytical studies

First Author, Year, Ref. of Meta-Analysis	Number & Type of Studies	Publication Year of Studies	Fresh red Meat RR	Processed Meat RR	Meat portion (g/d)	Excess Risk per 100 g	Ratio of RR/g Processed/Fresh Red meat
Larsson 2006 (6)	18 cohorts	1966-2006	1.22		120	+0.18	
				1.09	30	+0.30	1.64
Norat 2002 (5)	18 case-contr. + 6 cohorts	1973-1999	1.24		120	+0.20	
				1.36	30	+1.20	6.00
Sandhu 2001 (4)	13 cohorts	1980-1999	1.17		100	+0.17	
				1.49	25	+1.96	11.53

Table 2: Prospective studies published between 2003 and 2006, on the association between processed meat intake and colorectal cancer risk.

Author Publication Year	Localisation	Number of participants	Study Years	Type of meat	Adjusted Relative Risk	95% confidence interval	End-point
Oba et al., 2006	Japan	13,894 men	1992-2000	Processed meat : ham, sausage, bacon, yakibuta	1.98	1.24-3.16	colon cancer
		16,327 women			0.85	0.5-1.43	
Dixon et al., 2004	Europe	Re-analysis (ATBC, NLC and SMC)	1985-1992	Pork, Processed meat AND Potatoes	1.62	1.12-2.34	colon cancer
Fung et al., 2003	USA	76,402 women (Nurses' Health Study)	Several years	"Western pattern" (red/processed meat, sweets, desserts, French fries, refined grains)	1.46	0.97-2.19	colorectal cancers
Kesse et al., 2006	France	67,312 women (EPIC's French cohort)	1993-2000	"Western pattern" (processed meat, potatoes, pizzas, pies, sweets, cakes, cheese, eggs, butter)	1.39	1.0-1.94	Colorectal cancer
				"Drinker Pattern" (sandwiches, snacks, processed meat, alcoholic beverages)	1.42	1.1-1.83	

Table 3: Case-control studies published between 2003 and 2007, on the relationship between processed meat intake and colorectal cancer risk.

Name	Localisation	Number Case/Control	Date	Type of meat	Odd Ratio	95% confidence interval	End point
Chiu et al., 2003	China	931/1552	1990 - 1993	Preserved foods	2.0 ^b 2.7 ^a	1.5-2.9 1.9-3.8	Colon cancer
Chiu et al., 2004	USA	146/226	1994 - 1996	Processed meat	1.4 age 30 1.6 recent	0.7-2.5 0.9-3.1	Colorectal cancer
Nkondjock et al., 2004	Canada	202/429	1989 - 1993	"Pork processed meat pattern" (processed meat, pork and white bran)	1.6	0.9-2.85	Colon cancer
Murtaugh et al., 2004	US	952/1205	1997 - 2002	Processed meat	1.23 ^b 1.18 ^a	0.85 - 1.7 0.87 - 1.61	Rectal cancer
Kimura et al., 2007	Japan	782/793	2000 - 2003	Processed meat	1.15	0.83-1.6	Colorectal adenocarcinoma
Hu et al., 2007	Canada	1695/3097	1994 - 1997	Processed meat	1.6 ^b 1.5 ^a 1.4 ^b 1.5 ^a	1.0-2.4 1.0-2.3 1.0-2.0 1.0-2.2	Proximal colon cancer Distal colon cancer

a : RR in men,
b : RR in women