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MiniReview

Cancer Prevention Research

Heme Iron from Meat and Risk of Colorectal Cancer: A Meta-analysis and a Review of the Mechanisms Involved

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Abstract

Red meat and processed meat intake is associated with a risk of colorectal cancer, a major cause of death in affluent countries. Epidemiological and experimental evidence supports the hypothesis that heme iron present in meat promotes colorectal cancer. This meta-analysis of prospective cohort studies of colon cancer reporting heme intake included 566,607 individuals and 4,734 cases of colon cancer. The relative risk of colon cancer was 1.18 (95% CI: 1.06–1.32) for subjects in the highest category of heme iron intake compared with those in the lowest category. Epidemiological data thus show a suggestive association between dietary heme and risk of colon cancer. The analysis of experimental studies in rats with chemically-induced colon cancer showed that dietary hemoglobin and red meat consistently promote aberrant crypt foci, a putative precancer lesion. The mechanism is not known, but heme iron has a catalytic effect on (i) the endogenous formation of carcinogenic *N*-nitroso compounds and (ii) the formation of cytotoxic and genotoxic aldehydes by lipoperoxidation. A review of evidence supporting these hypotheses suggests that both pathways are involved in heme iron toxicity.
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Introduction

Cancer of the colon and rectum, taken together, are the third most common type of cancer worldwide (1). In most publications, colon and rectal cancer are studied together and the term colorectal cancer (CRC) is used, which we also use here, except when the publications refer specifically to colon or rectal cancer. CRC is the second most common cause of cancer death in affluent countries. Dietary modifications might reduce this cancer burden by up to 70% (2). Three recent meta-analyses showed that total meat intake is not related to risk but that intake of red or processed meat is associated with a modest, but significant risk of CRC (3–5). Processed meat intake appears to be more closely linked with the risk of CRC than fresh red meat intake. In its 2007 report, the World Cancer Research Fund panel recommended that one should limit intake of red meat and avoid processed meat (1).

Several mechanisms may explain the relationship between the risk of CRC and the intake of red or processed meat. First, meat cooked at high temperature contains mutagenic heterocyclic amines. But heterocyclic amines might not be major players in CRC risk, as: (i) consumption of chicken is a major contributor to intake of heterocyclic amines, but is not associated with the risk (6); and (ii) doses of heterocyclic amines that induce cancer in animals are 1,000 to 100,000 times higher than the dose ingested by humans (7). A second hypothesis suggests that the high saturated fat content of red and processed meat increases the risk of CRC. But several studies, including a recent meta-analysis, showed no effect of saturated fat on colorectal carcinogenesis (8–11). A third hypothesis concerns the carcinogenic *N*-nitroso compounds (NOC), which can be formed in the gastrointestinal tract by N-nitrosation of peptide derived amines or amides. The role of NOC in human cancer is discussed in the following text. Other more unlikely hypotheses involve the high protein, cholesterol, and salt content of red or processed meat. For a review of all these mechanisms, see ref. 12.

Sesink and colleagues suggested that heme iron, in the form of hemin [chloroprotoporphyrin IX iron(III)] a ferric form of heme, may explain the link between the risk of colon cancer and red meat intake, and the lack of a link with white meat intake (13). Epidemiological and experimental evidence support heme toxicity. Heme consists of an iron atom present at the center of a large heterocyclic organic ring called a porphyrin (Fig. 1). Heme is included in so-called hemoprotein, that is, hemoglobin, myoglobin (both involved in the oxygen supply), and in cytochromes (which catalyze electron transfer reactions). Red meat

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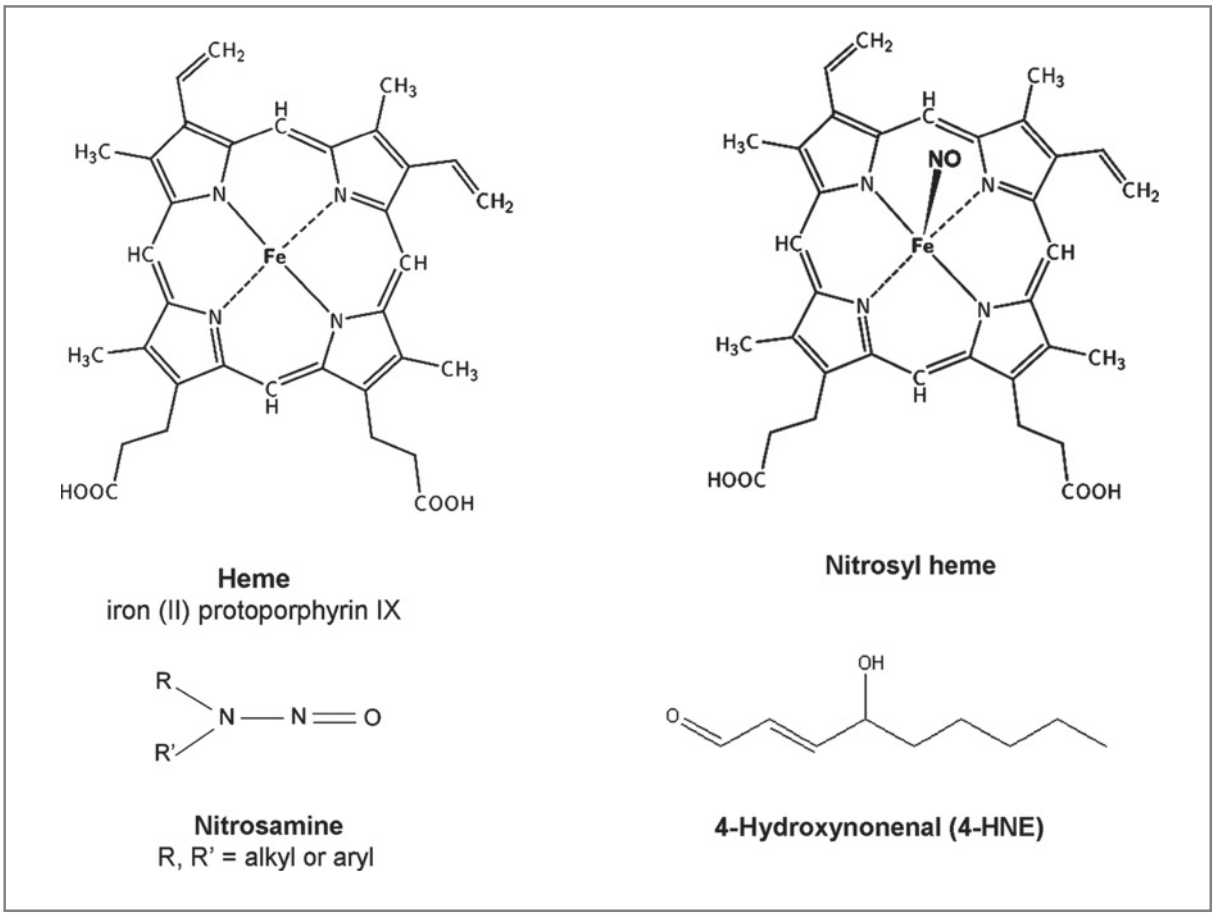

Figure 1. Structure of molecules cited in the review.

(such as beef, veal, lamb, mutton, pork, and offal) owes it dark red color to the presence of a high concentration of myoglobin, and the heme content of red meat is 10-fold higher than that of white meat (such as chicken; ref. 14). In processed red meat, heme iron is nitrosylated, because curing salt contains nitrate or nitrite (Fig. 1; ref. 12).

The aims of the present mini-review were: (i) to conduct a meta-analysis of epidemiological cohort studies on heme intake and the risk of colon cancer; (ii) to review experimental evidence supporting the aforementioned heme hypothesis; and (iii) to understand the mechanism of action of heme in carcinogenesis.

Heme iron intake and risk of colon cancer: a meta-analysis of prospective cohort studies

The objective of this part of the review was to assess, through meta-analysis, the magnitude of the relation between heme iron intake and colon cancer. As most studies do not report data on rectal cancer, we decided to limit our analysis to colon cancer. The methodological procedure is described in the Supplementary Material to this article.

The characteristics of the 5 prospective cohort studies included in the meta-analysis are summarized in Supplementary Data (Table S1). This meta-analysis included data on 566,607 individuals and 4,734 cases of colon cancer. Although 1 cohort study found no association between heme and cancer (15), 3 found that a high intake of heme iron was linked with a higher risk of colon cancer (16–18), and 1 found a positive, but not significant, association between heme iron and colon cancer (19; Fig. 2). In the Lee and colleagues study, the relative risk (RR) for both proximal and distal colon was 1.53 (95% CI: 0.99–2.38). In the Balder and colleagues study, the association was positive in the 2 genders combined (RR = 1.35, 95% CI: 1.03–1.77; ref. 17). The summary RR of colon cancer in all 5 studies was 1.18 (95% CI: 1.06–1.32) for subjects in the highest category of heme iron intake compared with those in the lowest category (Fig. 2). This meta-analysis showed a consistent association between high intake of heme iron and increased risk of colon cancer.

Two studies out of 5 considered calcium in the adjustments for the RR (16–18), and showed the strongest

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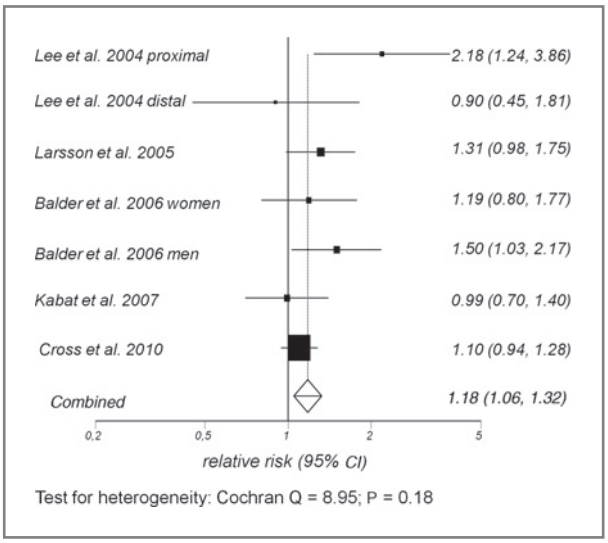

Figure 2. Relative risks of colon cancer in prospective cohort studies, comparing the highest with the lowest category of heme iron consumption. Studies are ordered by year of publication. Squares represent study-specific RR and the size of squares is proportional to the statistical weight that each contributed to the summary estimate of relative risk (percentage weight of each study: Lee *et al*., 2004, proximal: 6.6%; Lee *et al*., 2004, distal: 4.6%; Larsson *et al*., 2005: 18%; Balder *et al*., 2006, women: 11.7%; Balder *et al*., 2006, men: 12.86%; Kabat *et al*., 2007, 14.2%; Cross *et al*., 2010: 32%). Horizontal lines represent 95% CI. The diamond represents the summary estimate of the relative risk of all studies included in the meta-analysis.

association between heme iron and colon cancer. This makes sense, as calcium inhibits heme-induced cytotoxicity, colonic epithelial hyperproliferation, and promotion of chemically induced carcinogenesis in animal models (20–22).

Two studies we excluded from the meta-analysis found similar results. An ecological study found a direct correlation between the dietary iron index and colon and rectal cancer (23). Ferrucci and colleagues observed a positive, but not significant, association between heme iron in diet and colorectal adenoma.

The present meta-analysis is the first to examine the relation between heme iron and colon cancer. But this study also has its limitations; first it includes only 5 cohort studies, and the way heme intake was measured differs in each study. Lee and colleagues and Larsson and colleagues calculated heme iron content in the diet by applying a factor of 0.4 to the total iron content of all meat items which essentially is reporting an overall red meat effect (16, 18). Balder and colleagues multiplied the heme iron content of each meat item by the mean daily intake of the relevant food item, estimated from the Dutch Food Composition Database (17), but the 2 methods yielded similar results (15). Cross and colleagues developed a new heme iron database based on measured values in conjunction with a detailed meat cooking questionnaire (19).

In conclusion, this meta-analysis showed a significant and consistent but modest increase in the risk of colon cancer associated with high heme iron intake. This study should be pursued by future prospective cohort studies, but this epidemiological result is in line with experimental *in vivo* results detailed in the following text.

Experimental evidence of colorectal cancer promotion by heme iron

Sawa and colleagues showed that dietary hemoglobin produces lipid peroxyl radicals and increases the incidence of nitrosomethylurea-induced colon cancer in rats fed polyunsaturated fat (24). Sesink and colleagues studied the effect of hemin-supplemented diet in non-initiated rats. Dietary hemin increases fat peroxidation and cytotoxic activity of fecal water, and epithelial proliferation by 70% (13). In hemin, the iron atom is stabilized by a freely exchangeable chloride. Pierre and colleagues also showed that hemin and hemoglobin increase the number of azoxymethane-induced aberrant crypt foci, which are putative preneoplastic lesions, in the colon of rats (21). In contrast with hemin, dietary hemoglobin does not increase the cytotoxicity of fecal water, and it is less potent than hemin in promoting colon carcinogenesis. Hemoglobin may be a suitable substitute for myoglobin in nutritional experiments with animal model, and a model agent for studies on the cytotoxicity of red meat (21).

Pierre and colleagues also fed 3 types of meat with different heme content (chicken, beef, and blood sausage) to rats treated with azoxymethane and fed a low-calcium diet (25). This study was the first to show that dietary meat can promote colon carcinogenesis, and that the effect depends on the heme concentration. The results of this study of meat contrast with those of several earlier studies, where red-meat based high-calcium diets failed to promote colon carcinogenesis, indicating probable protection by calcium (26). Subsequently, Pierre and colleagues tested the hypothesis, suggested by epidemiology, that nitrosyl heme in processed meat was more toxic than native heme in fresh meat (27). Cured meat can indeed promote colon carcinogenesis in rats (27). Dietary hemin, but not hemoglobin, could be used as a model agent to mimic the effects of processed meat in rats (27). In a recent study, Pierre and colleagues demonstrated that the nitrosylation of heme was a key event in the promoting effect of processed meat in rats (28).

Analysis of the results of experimental studies of rats with chemically-induced colon cancer (21, 22, 25, 29), showed that the global standardized effect size for number of aberrant crypt foci per colon was 1.73 (95% CI: 1.33–2.14) in rats given dietary heme iron in hemoglobin or beef meat, compared with control rats. The logistic regression approach showed a significant correlation between the number of aberrant crypts per colon and the concentration of heme in the diet ($P = 0.02$; see Methods and Figure in Supplementary Data). This experimental evidence that heme iron promotes carcinogenesis in rats is consistent

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with epidemiological evidence. Heme promotion may explain why the intake of red and processed meat is associated with a risk of CRC.

Possible mechanisms of heme toxicity in the gastrointestinal tract

The mechanisms implicated in the promotion of colorectal cancer by heme are poorly understood. The mechanistic hypotheses are based on the catalytic effect of heme iron on (i) the formation of NOC and (ii) the formation of lipid oxidation endproducts.

Heme iron catalyzes N-nitrosation

NOC are formed by N-nitrosation of amines and amides, produced primarily by bacterial decarboxylation of amino acids in the presence of a nitrosating agent (30). There was no *a priori* reason to think that nitrosation would require heme iron. The structure of nitrosamine is shown in Figure 1. NOC can be detected by thermal energy analysis following the release of nitric oxide from biological samples. This analytical procedure comprises nitrosyl iron and *S*-nitrosothiols in addition to nitrosamines and nitrosamides, which are collectively referred to as apparent total *N*-nitroso compounds (ATNC; ref. 31).

Animal and human studies. Bacon-fed rats had a fecal concentration of ATNC 10 to 20 times higher than control rats (32). In addition, mice fed a diet of hot-dogs (18%), had 4 to 5 times more ATNC, and mice fed a beef diet had 2 to 3 times more ATNC in their feces than controls fed no meat (33, 34).

Human volunteers given a high red meat diet excreted much more ATNC in their stools than controls given no or little red meat, or only white meat (31, 35, 36). The fecal concentration of ATNC was 60 times higher in volunteers given cured red meat than in volunteers given a vegetarian diet (37). Heme iron, and not inorganic iron or meat proteins, may be responsible for the nitrosation observed in the gut of volunteers fed red meat (38).

Nature of ATNC. A red meat diet increased nitrosyl iron and nitrosothiols in ileal outputs and in stools of volunteers, compared with a vegetarian diet, suggesting that these compounds contribute significantly to ATNC (39, 40). Nitrosothiols are rapidly formed from nitrite and thiol groups at low pH in the stomach and can be precursors for the formation of nitrosyl heme and NOC in the gut (39). The strong correlation between fecal nitrosyl iron and fecal heme suggests that nitrosyl heme is the main source of nitrosyl iron (39). Moreover, ATNC precursors from hot dogs were partially purified and separated by HPLC (41). One fraction was identified as 1-deoxy-*N*-1-glucosyl glycine by mass spectrometry, and the nitrosated fraction was shown to be mutagenic by the Ames test (41).

Carcinogenicity of nitrosated compounds. The carcinogenicity of ATNC formed in the gut after eating heme from red or processed meat is unknown. Parnaud and colleagues found no initiation or promotion of preneoplastic lesions by ATNC in the colon of rats fed a bacon-based diet (32). Kunhle and colleagues speculated that nitrosyl iron compounds and nitrosothiols may contribute to the tumorogenic potential of the diet (39). By contrast, in a commentary on Kunhle's article, Hogg speculated that the sequestration of the "nitrosating potential" of the diet as nitrosothiol or as nitrosyl iron may be a protective mechanism that would limit the formation of DNA alkylating agents (42).

However, several arguments suggest that ATNC may be important genotoxins. First, most NOC, such as nitrosamines, nitrosamides, and nitrosoguanidines, can yield alkylating agents during metabolism, and cause DNA damage. For instance *N*-methyl-*N*-nitrosurea intrarectally perfused induced G → A transitions in K-*ras* in 30% of rat colon carcinoma (43). In addition, nitrosated glycine derivatives reacted with DNA to give rise to promutagenic and toxic adducts including O^6-methylguanine and O^6-carboxymethylguanine (44). O^6-Carboxymethylguanine adducts were found in stool exfoliated colonocytes from volunteers eating red meat, with a correlation between the level of adducts and of fecal ATNC, suggesting that ATNC are genotoxic (45). Moreover, potassium diazoacetate, a stable form of nitrosated glycine, was shown to induce mutations in the *p53* gene in a functional yeast assay (46). The patterns of mutations were similar to the patterns observed in human colon tumors. This supports the hypotheses that nitrosation of compounds related to glycine contributes to *p53* mutations in humans, and that O^6-carboxymethylguanine adducts in exfoliated colorectal cells are related to CRC (46).

Heme iron catalyzes the oxidation of polyunsaturated fats

The polyunsaturated fatty acid residues of phospholipids are extremely sensitive to oxidation. Lipid peroxidation is initiated by free-radical attack of membrane lipids and is catalyzed by heme with the following reaction: LOOH (lipid hydroperoxide) + Fe-ligand (heme) → LOOFe ligands → $LO^{\cdot}$ (lipid alkoxy radical) + $^{\cdot}$OFe ligands (heme oxiradical; ref. 47). The initial products of unsaturated fatty acid oxidation are lipid hydroperoxides, but they are relatively short lived. They are either reduced by glutathione peroxidase to unreactive fatty acid alcohols or they react with metals to produce a variety of reactive compounds such as epoxides and aldehydes. The major aldehyde products of lipid peroxidation are malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE; ref. 48). These dietary lipid oxidation end products are risk factors for several human diseases (for review, see refs. 49, 50).

Malondialdehyde. MDA is formed by oxidation of polyunsaturated fatty acids with 2 or more double bonds. MDA-induced DNA damage is mutagenic in bacterial, mammalian, and human cells (51–53). MDA reacts with DNA to form adducts with deoxyguanosine, deoxyadenosine, and deoxycytidine (for review, see ref. 54). The major

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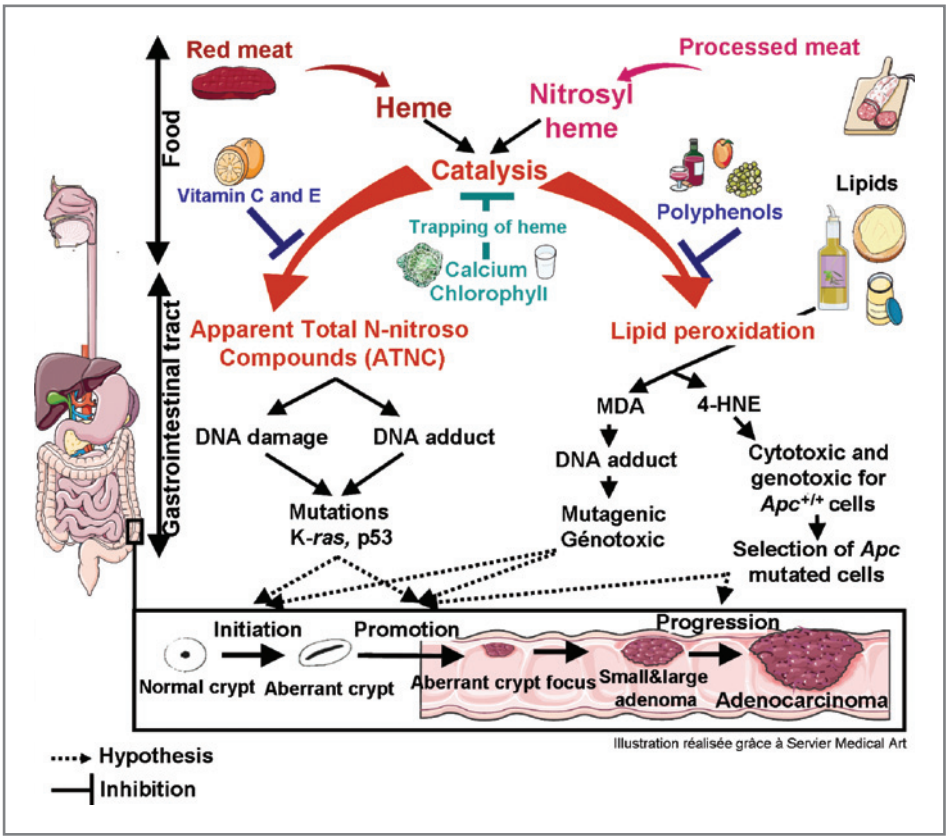

Figure 3. Catalytic effects of heme on the formation of ATNC and lipid peroxidation, and their inhibition. Consequences for the development of CRC. Heme catalyzes the formation of ATNC and lipid peroxidation endproducts, which partially explains the promoting effect of red and processed meat on CRC. The catalytic effects of heme can be inhibited by trapping the heme (calcium, chlorophyll). The endogenous formation of ATNC is inhibited by vitamins C and E, and it appears that polyphenols can inhibit lipid peroxidation.

DNA adduct formed by reaction of MDA with DNA is 1,N^2-malondialdehyde-deoxyguanosine (M_1dG). M_1dG was detected in colorectal biopsies from normal mucosa of 162 participants in the United Kingdom FlexiScope Sigmoidoscopy Screening Trial and the EPIC study (55). The level of this adduct was modulated by dietary and lifestyle habits, and there is to higher M1dG levels in subjects with adenoma compared with adenoma-free subjects ($P < 0.005$; ref. 55).

4-Hydroxynonenal. In contrast with MDA, 4-HNE is weakly mutagenic but appears to be the main toxic product of lipid peroxidation (Fig. 1). 4-HNE has powerful effects on signal transduction pathways and some of its effects appear to be independent of DNA damage (48). Indeed, 4-HNE present in fecal water can induce apoptosis and necrosis of human colon carcinoma cells through caspase 3 activation (56). Mutations in the *adenomatous polyposis coli* (*Apc*) gene on the chromosome *5q21* locus are considered to be one of the earliest events in the initiation of CRC (57). Moreover, *Apc* mutation was shown to reduce the level of caspases 3, 7, and 9 in mouse colonocytes, leading to resistance to apoptosis (58). An intestinal cell line derived from C57BL/6J mice ($Apc^{+/+}$) and Min mice (*Apc* Min/$^+$) retained the heterozygous Apc genotype and the disordered actin cytoskeleton network for the *Apc* Min/$^+$ cell line (59, 60). By exposing this cell line to fecal water of heme-fed rats or to 4-HNE, Pierre and colleagues showed that apoptosis was suppressed in *Apc* Min/$^+$ cells (61). The heterozygote *Apc* mutation is thus a strong selective advantage for colonic cells exposed to a lipoperoxidation-related genotoxic environment such as excess heme iron or 4-HNE (61).

In summary, heme catalyzes the formation of ATNC and of lipid oxidation end products, which may explain the promoting effects of red and processed meat on CRC. However, the procarcinogenic effect of heme can be inhibited by several molecules. First, calcium salts and chlorophyll can precipitate heme molecules and inhibit the cytotoxic and hyperproliferative effect of heme in the rat epithelium (17, 20–22, 62, 63). Moreover, the endogenous formation of ATNC is inhibited by vitamins C and E, and lipoperoxidation is inhibited by several polyphenols such as quercetin, α-tocopherol, or red wine polyphenols (64–68). The catalytic effects of heme and its inhibition are summarized in Figure 3.

Conclusion

CRC is the leading cause of cancer death among nonsmokers in affluent countries, and its prevention is thus a major goal for public health. Epidemiological studies

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demonstrate a modest but significant and consistent relation between red meat and processed meat intake and CRC risk. The dietary recommendations are to reduce red meat intake and to avoid processed meat intake (1). However, meat is an important source of proteins, providing all essential amino acids, and it is an excellent source of iron and zinc. Iron deficiency is the most widespread nutritional disorder in the world, especially among children and premenopausal women, and results in iron deficiency anemia (1). Knowledge of the mechanism of CRC promotion by meat may allow an alternative prevention strategy to be developed: inhibiting red and processed meat toxicity instead of stopping meat intake. Among the hypotheses explaining the association between meat intake and the risk of CRC, the effect of heme iron is supported by both epidemiological (Fig. 2) and experimental evidence (Supplementary Fig. S1). Several mechanisms may explain the effect of heme on CRC, and the 2 major hypotheses are: (i) heme catalyzes the endogenous formation of ATNC; and (ii) heme catalyzes the peroxidation of dietary fats (Fig. 3). Calcium salts, chlorophyll, vitamin C, and several polyphenols may reduce these deleterious effects of heme. Specific recommendations might be made, for example, "eat a yogurt after your steak." Moreover, vitamins or polyphenols could be added during the curing process. Ascorbic acid is already added during the processing of processed meats specifically to inhibit the formation of volatile NOC in the meat (69). We expect that this will reduce the risk of CRC without losing the benefit and the pleasure of eating meat.

Disclosure of Potential Conflicts of Interests

No potential conflicts of interests were disclosed.

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