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Meat and Colorectal Cancer: Associations and Issues

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Abstract Epidemiologic studies indicate an association of modest strength between consumption of red meat and colorectal cancer risk. Candidate compounds in red meat implicated in this association include those derived from processing (heterocyclic aromatic amines [HAAs], polycyclic aromatic hydrocarbons [PAHs], and N-nitroso compounds [NOCs]), as well as heme. Questions regarding HAAs and PAHs as etiological agents include their low concentration in meat relative to high concentrations in experimental studies and differing colorectal associations between different HAA and PAH food sources. The role of added nitrite and nitrate meat preservatives in NOC formation, as well as the potential inhibitory effect of calcium on heme-stimulated NOC formation remain uncertain and warrant further investigation. Improvements in dietary exposure assessment methods for the exogenous compounds and a greater understanding of gene–diet interactions will be necessary to clarify the role of meat mutagens and to more firmly establish the relationship between meat consumption and colorectal cancer.

Keywords Cancer risk · Cancer prevention · Colorectal cancer · Meat · Processed meat · Heterocyclic aromatic amines · Polycyclic aromatic hydrocarbons · *N*-nitroso compounds · Heme iron · Saturated fat · Calcium

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Introduction

Accounting for approximately 10 % of all cancers, colorectal cancer is the third most common cancer in men, and second most common cancer in women worldwide [1]. In addition to several internal factors (e.g., genetic variation), many external and lifestyle factors have been investigated as causal factors, with diet receiving much attention, especially meat. Due to key differences in composition, meat is often sub-categorized into the following groups: red meat (animal flesh with a higher proportion of red versus white muscle fibers), which includes beef, goat, lamb and pork; white meat (with more white versus red muscle fibers), such as flesh from poultry, fish, and domestic rabbit; and processed meat, which is more loosely defined but generally means red or white meat that has been preserved through salting, curing, smoking, or the addition of preservatives (e.g., ham, bacon, sausage, hot dogs, sandwich meats). In 2007, the World Cancer Research Fund and the American Institute for Cancer Research (WCRF/AICR) concluded that the quality of the evidence was convincing (their highest quality category) for a positive association between red and processed meat and colorectal cancer [2]. In 2011, the WCRF/AICR published their Continuous Update Project report on colorectal cancer, showing that studies since 2007 continue to support and strengthen the conclusion of a convincing positive association with red and processed meat [3].

Despite the publication of these two seemingly conclusive reports based on rigorous review and assessment processes, well over 25 reviews of meat and colorectal cancer have been published in the last 5 years. These reviews, taken together, generally support a positive association between meat and colorectal cancer. However, some still question this association and several others question the proposed mechanisms behind meats increasing colorectal cancer risk. Because red meat is rich in key nutrients, an overarching concern, either stated directly by many of these review authors or implied, is

that if intake of meat is limited to an unnecessarily low level, this could lead to negative health consequences, particularly to the large number of people with existing nutrient deficiencies (e.g., iron deficiency). There is evidence of over-interpreting the WCRF/AICR findings into recommendations of replacing red meat with white meat and fish [4], a primary concern that was clearly articulated by Oostindjer et al. [5••]. Meat, and particularly red meat, is an excellent source of easy-to-absorb iron, zinc, selenium, polyunsaturated fatty acids, and B vitamins, with B_6 and B_{12} of particular note. Without a clearer understanding of the association between red meat and colon cancer, and the mechanism(s) behind this association, important opportunities may be missed to improve the modifiable quality of meat and meat products in ways that maximize health benefit and eliminate cancer risk. Hence, herein we present the key issues raised in the research literature regarding the meat and colorectal cancer association that need to be addressed for meaningful progress in decreasing colorectal cancer incidence and maximizing healthy dietary options. We will discuss the issues as divided into two broad categories: those related to the proposed mechanisms and those related to methods used in studying meat and colorectal cancer.

Issues with Proposed Mechanisms

Several mechanisms have been postulated as to how red and processed meats could lead to colorectal cancer. These can be categorized into mechanisms related to 1) compounds not naturally inherent to meat, but that are formed or added during cooking and processing; and 2) compounds that are naturally inherent or endogenous to meat and meat products.

Compounds Generated by Cooking and Processing

Heterocyclic Aromatic Amines When meat (of any type) is cooked at high temperatures or for prolonged periods of time, Maillard reactions take place between creat(in)ine, amino acids, and sugars to produce heterocyclic aromatic amines (HAAs). Numerous animal studies report colon carcinogenesis after administration of various HAAs (reviewed in [6]). Furthermore, they are classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans [7]. Two related issues have been raised regarding the role of HAA in colorectal cancer. First, the amounts of HAAs used in animal studies are exceedingly high relative to levels in human food or what could be achieved through the human diet [8, 9]. While this may be due in part to interspecies differences in metabolism and susceptibility to HAAs, or the potential synergy of these compounds in the human diet compared with administration of a single or few HAAs in animal studies, it has been pointed out that epidemiologic studies of HAAs and colorectal cancer have been inconsistent [9]. Second, an additional question stems from the seeming paradox of grilled or fried chicken being as high (or higher) in HAAs than similarly prepared red meat, and yet it is not associated with colorectal cancer [8, 10]. Although a proposed explanation for this is that HAAs generated in beef may be more potent than those generated in chicken [8], it is not clear whether this has been investigated adequately.

Polycyclic Aromatic Hydrocarbons The incomplete combustion of organic compounds produces polycyclic aromatic hydrocarbons (PAHs), which can be found in cooked meats; in particular, pyrolysis of fats from cooking meat over an open flame leads to PAH formation [11]. Benzo[a]pyrene (BaP) is the most investigated PAH, and according to IARC monographs, BaP is classified as carcinogenic to humans; however, evidence of carcinogenicity from dietary exposures is inconclusive [12]. As with HAAs, some contend a similar paradox warranting investigation: chicken and fish are sources of PAHs when grilled or fried, yet these meats are not associated with colorectal cancer [5••]. It has also been pointed out that levels of PAHs from meat are relatively low in the human diet [13]. Interestingly, a 2014 review of meat and colorectal cancer explained that atmospheric deposition of PAHs results in contamination of grains, cereals, and vegetables with PAHs [14••]. It went on to report that a study showed grains/bread/cereals and grilled/barbecued meat contributed 29 % and 21 %, respectively, to daily human intakes of BaP [15]; however, there was no discussion as to why meat BaP would result in colorectal cancer and BaP from grains/bread/cereals would not. Unless the diet is high in smoked or barbecued meats, it is cereals and vegetables, and their fats and oils, that are proposed as the major contributions to human dietary BaP exposure [11].

N-nitroso Compounds Among other things, nitrates and nitrites are used as fertilizers on crops, which can result in high concentrations in some foods [16]. Of greater concern is the use of nitrates and nitrites as food preservatives, particularly in cured meats (e.g., hot dogs, bacon, lunch meats). Once ingested, nitrate is reduced to nitrite by the microflora in the digestive tract and nitrites react with nitrosation precursors such as amines and amides to form N-nitroso compounds (NOCs). They have been classified as probably carcinogenic to humans by IARC [17]. Compared with HAAs and PAHs, there is seemingly less controversy regarding NOCs and the potential role of nitrates and nitrites in colorectal cancer. The issues that are raised regarding these compounds are: 1) does the association with colorectal cancer truly include red meat or is it just processed meats treated with nitrates and nitrites? 2) If the association is with processed meats and subsequent NOCs, then are there ways to overcome negative NOC effects? The first question is a matter of classification that will be addressed in the discussion of methodological issues. The second can be viewed as an opportunity that has not been sufficiently

investigated. Lund et al. call attention to the European Prospective Investigation into Cancer cohort, which showed that the impact of meat on colorectal cancer risk was minimal in those with high fiber and fish-rich diets [10]. They posit that there is the potential for negative effects of processed meats to be counteracted with the inclusion of leafy vegetable consumption. Others strongly suggest a need for greater exploration of methods to improve processed meat composition or the composition of the overall diet, such as by adding calcium or chlorophyll or by replacing nitrites with phytochemicals in order to reduce or eliminate the risk related to nitrates and nitrites [5••, 8].

Endogenous Compounds

Several different compounds endogenous to meat have been postulated to increase colon cancer risk. These include total iron, heme iron, saturated fatty acids, and protein fermentation products. However, there is little experimental support for a role of non-heme iron [18, 19] or for fat from meat [20, 21] in colon carcinogenesis. Although protein fermentation products appear to be toxic to the colonic epithelium, their role in promoting colon cancer remains uncertain [22]. Therefore, in this section, we shall focus on the role of heme in colon carcinogenesis.

Epidemiology Although many epidemiological studies have examined the association between meat, meat products, and colon cancer risk, few have specifically examined the association between heme iron intake and colon cancer risk. A 2013 meta-analysis of prospective cohort studies reported a relative risk (RR) of 1.14 for subjects in the highest heme intake category compared to the lowest (95 % CI: 1.04–1.24) [23]. In 2014, a meta-analysis of prospective studies of heme iron intake and colon cancer risk reported a RR of 1.12 (95 % CI: 1.03–1.22) [24]. Since both meta-analyses were analyzing essentially the same studies, the similar relative risks are not surprising. Also, both had the same challenge of estimating heme intake, as the heme iron content of meat varies substantially with the type of meat (e.g., beef vs. pork vs. poultry) and the method of preparation [25•]. Several of the studies examined in the meta-analyses estimated heme iron intake by assuming a heme iron content of 40 % of total iron, which would certainly introduce error into the estimate of heme iron intake, as actual values vary from 33 to 75 %. Given the modest increase of 12–14 % in colon cancer relative risk with greater heme consumption, the difficulty in estimating actual heme iron intake, and the potential confounders of the exogenous compounds described above, these findings should be viewed cautiously.

In a recent study, a molecular genetic epidemiological approach was used to understand the association between heme iron intake and colorectal cancer risk by examining specific mutations in colon tumors in the adenomatous polyposis coli (*APC*) tumor suppressor gene and Kirsten ras (*KRAS*) oncogene [26]. In this nested case-control study, no difference was found between cases and controls in daily heme intake in women. In men, there was a greater heme intake in colon cancer cases compared to controls, but a lower heme intake in rectal cancer cases. However, there was a significant trend for an increase in the hazard ratio between the lowest and highest tertile of heme iron intake and greater numbers of activating/truncating G>A mutations in either *KRAS* and/or *APC* in colorectal tumors, although not with activating G>T mutations or in tumors with neither mutation. There was no association between heme iron intake and number of tumors without these aberrations. Since G>A transitions are known to be characteristic of DNA alkylating agents [27], the authors speculate that heme iron is acting to induce these alkylating events, possibly by stimulating formation of NOCs, which have been implicated in inducing alkyl DNA adducts. This study suggests that heme intake is associated with specific types of mutations or alterations in expression of cancer-related proteins in the colon, and may thus provide insight into a mechanistic understanding of the association between heme and colon cancer risk.

Animal Studies To date, there appear to be no studies of humans fed isolated heme or heme-containing proteins to investigate the influence of heme on markers of colon cancer. Surprisingly, few animal studies have examined the effect of heme, in isolated form, on colon cancer. Two studies conducted by Pierre et al. examined the effect of isolated heme or hemoglobin on colonic precancerous lesions (aberrant crypt foci, ACF). In the first, rats treated with the colon carcinogen azoxymethane were fed different dietary concentrations of hemin or hemoglobin for approximately 100 days [28]. Aberrant crypt number increased in a dose-dependent manner with increasing dietary concentrations of hemin, from 0.25 μmol/g to 1.5 μmol/g. However, heme derived from hemoglobin, at a dietary heme concentration of 1.5 or 3.0 μmol/g, increased the aberrant crypt number only modestly. It should be noted that these are somewhat high concentrations of heme, as hamburger contains approximately 0.5 μmol heme/g dry wt. [25•]. Thus, the dietary heme concentrations used in this study are not possible to obtain by a normal diet. In a second study [29], hemoglobin was fed to azoxymethane-treated rats at a dietary heme concentration equivalent to a diet containing 60 % beef for 100 days. Colons of rats fed hemoglobin had a statistically significant 29 % increase in ACF and a 336 % increase in mucin-depleted foci (MDF), highly dysplastic ACF that are considered more likely than ACF to progress to tumors [30]. These two studies, taken together, suggest that 1) heme at high dietary concentrations promotes formation of precancerous lesions in the colon of carcinogen-treated rats; and 2) hemin is more

carcinogenic than hemoglobin. It would be of interest to see whether hemoglobin, the major food form of heme, promotes colon carcinogenesis when fed at a dietary concentration similar to that consumed by individuals who consume meat.

Possible Mechanism of Action There are two primary hypotheses to explain how heme may increase the risk of colon cancer. One is that heme results in production of reactive oxygen species (ROS) in the lumen of the colon. As discussed by Tappel [31], heme catalyzes oxidative reactions, the products of which can damage lipids, proteins and DNA. Thiobarbituric acid reactive substances (TBARS), a measure of lipid oxidation, was approximately doubled in the fecal water of mice fed diets containing either 0.2 or 0.5 μmol heme/g diet for two weeks [32]. Although it has long been speculated that a high production of oxygen radicals within the colonic lumen may promote colon cancer [33, 34], this connection remains to be established. Indeed, the oxygen radical absorbance capacity (ORAC), a measure of antioxidant capacity, of the cecal contents supernatant does not correlate with colonic ACF number in carcinogen-treated rats [35]. Since a high ORAC would be expected to reduce oxygen radicals, this finding does not support a role for oxygen radical production in the colon lumen in colon cancer risk. Thus, although high dietary concentrations of heme clearly increase oxidative stress with the colonic lumen, the evidence of a link with colon cancer is uncertain.

Heme can stimulate formation of NOCs in the feces [18]. As discussed above, NOCs have been classified as probable carcinogens in humans. In vitro, nitrosated glycine compounds incubated with DNA give rise to both O^6-MeG and O^6-carboxymethylguanine (O^6-CMG) adducts [36]. In mice treated with the colon carcinogen and alkylating agent dimethylhydrazine, the number and persistence of O^6-methylguanine (O^6-MeG) adducts formed in the colon correlated with expected tumor number [37]. Thus, NOCs produced endogenously may increase cancer risk by producing promutagenic adducts. A recent study examined the effect of the digesta of different meats subjected to a simulated digestion on DNA adduct formation in vitro. The heme content of the different meats somewhat correlated with the number of O^6-CMG adducts formed, although the results varied considerably with the donor of the fecal inoculum used in the colonic stage of the simulator [38]. Since O^6-CMG adducts are viewed as specifically formed by NOCs, these findings were viewed as confirming heme-induced DNA adduct formation from NOCs. However, whether increased O^6-CMG adduct formation increases tumorigenicity seems uncertain. A recent study examined this question in mice using the western diet model of spontaneous colon cancer [39]. Mice were fed heme (as hemin) for 18 months and then colons examined for O^6-CMG adducts and tumor formation. Although the mice fed heme had a greater number of O^6-CMG adducts relative to mice fed the heme-free control diet, the number of tumors did not differ between the two groups [40••]. A notable feature of this study was the use of a dietary heme concentration that could plausibly be consumed in a human diet. Although this study does not support an increase in colon tumorigenesis by heme consumption, further examination of the influence of heme on DNA adducts and its correlation with more direct cancer markers, using different animal models, seems warranted.

Given the evidence suggesting that dietary heme promotes colon cancer, there has been interest in determining whether other dietary components may counteract this effect. Increased dietary calcium has been explored in this regard. In carcinogen-treated rats fed a diet high in heme, those fed a high calcium diet had significantly fewer MDF than those fed a low calcium diet [28], suggesting a protective effect of calcium. A more recent study examined carcinogen-treated rats fed a low calcium diet containing hot dogs as a source of heme, with or without additional calcium (as calcium carbonate) [41]. Rats fed the additional calcium had significantly fewer MDF, a lower fecal concentration of NOCs, and a much lower concentration of TBARS in the fecal water, indicating less lipid peroxidation, than rats fed the low calcium diet. In human subjects consuming 180 g/day of pork for 4 days, with or without 1 g/day of calcium supplementation, subjects consuming the supplement had a lower concentration of NOCs and TBARS in their fecal water [42]. Thus, adding calcium to a heme-containing diet reduces formation of NOCs and lipid peroxidation products in the large intestine. How might calcium be acting to produce these effects? In vitro, calcium phosphate reduces heme solubility, and high calcium diets increase fecal excretion of heme [43]. The most likely explanation is by decreasing the solubility of heme within the small intestine, the likely site of NOC formation [44, 45], thus decreasing its effective concentration.

Overall, with regards to the endogenous compound heme and the exogenously sourced compounds that are generated or added through various cooking and processing methods, a general cautionary tale is raised regarding the difficulty of assigning risk unequivocally to one group of compounds, and the difficulty of explaining the impact of diet factors in isolation [5••, 13, 46–49]. These authors remind us of the reality that other carcinogens and chemoprotectants, which the above groups of compounds could interact with, i.e., dietary components exist in matrices with other risk-modifying nutrients, are simultaneously present in the diet. A summarizing and overarching suggestion across many of the reviews over the past 5 years is that the risk posed by red and processed meat mostly certainly depends on the total diet and meal composition, as well as composition of the meat itself, which can be modified through changes in feed and processing [5••].

Issues Related to Methods

The complexity of diet, as alluded to above, leads to several challenges in conducting studies of diet effects on disease. It has been observed that, although a positive association has been found between red and processed meat and colorectal cancer, it is a weak association with relative risks less than 2.0, and the dose response could be interpreted as nonlinear rather than linear [5••, 9]. Several key methodological issues have been pointed out in the literature, which need to be overcome in order to clarify the association and mechanism.

Assessment of Correct Dietary Exposures

Two highly impactful discrepancies in epidemiologic studies of meat and colorectal cancer are how red and processed meat are defined (i.e., no uniform classification/categorization) and the frequent grouping of the two together [13, 14••, 50], despite the great variability in the composition of red meat and processed meat. Further, the type or definition of the dietary exposure, analytical cut-points of exposure, and the method by which exposure categories are analyzed, vary greatly [9, 13]. Despite HAAs and PAHs being proposed as mechanisms behind the red meat and colorectal cancer association, few studies actually assessed methods of cooking and levels of doneness [13, 50]. To address this, databases have been developed in recent years for estimates of HAA, BaP, nitrite, and nitrate content of foods [14••]; however, such databases need to be continually updated, and ways to address the large variability between pieces of meat due to breed, trimming, feed, etc., need to be developed [5••]. Similarly, as mentioned earlier, few studies have specifically assessed heme in human studies.

The complexity of the diet and human behavior raises the likelihood that meat intake in studies reflects more a dietary pattern (rather than meat per se [46, 51]) that needs more definitive elucidation and characterization. Initial efforts have been made in studying dietary patterns, but a key concern that is raised is reproducibility with current approaches [51]. Frequently, a need for caution has been voiced in interpreting studies showing red and processed meat positively associated with colorectal cancer because of other diet, lifestyle, and behavioral choices that could be associated with high meat intake; examples include low vegetable or antioxidant intake, which may result in confounding that cannot easily be accounted for [9, 48, 49, 52]. Similarly, animal studies may not have been interpreted with the complexity of diet in mind. Oostindjer et al. suggest that the animal data so far indicate less of an effect of meat on colorectal cancer and more of an effect of meat in diets without vegetables or cereal fibers, and which are often low in calcium [5••].

Underlying Gene–Diet Interactions

Many of the reviews in the past 5 years comment on the inconsistency in the epidemiologic literature examining the role of red and processed meat in colorectal cancer. While some of the inconsistencies could be due to exposure assessment issues as described above, there could also be genetic polymorphisms among the populations or subjects studied that result in differing responses and long-term consequences to meat intake. For example, some in the population may be more susceptible to HAAs and PAHs than others due to genetic variation related to metabolism of these compounds. A call for more studies of potential modification of the meat risk by genetic polymorphism is seen in some of the recent meat and colorectal cancer reviews [8, 9, 13, 50]. There is indeed growing interest in and ability to investigate whether there are significant gene-meat-colorectal cancer interactions. From 2010 to 2014, approximately 30 primary research papers were published on the results of investigating these interactions. It is beyond the scope of this review to summarize this body of work. However, the list of genes and their polymorphisms investigated so far represent a fascinating range of pathways directly and indirectly related to colorectal carcinogenesis. These pathways or mechanisms (and genes) include phase I and phase II biotransformation or xenobiotic metabolism (e.g., *CYPs*, *NAT*s, *UGT*s); phase III biotransformation or transport (e.g., *MDR1*, *BCRP*); transcription factors (e.g., *NFκB*, *PXR*, *LXR*); DNA repair (e.g., *XPA*, *XPC*, *XRCC1*, *APEX1*, *PARP*); mediators of intestinal inflammation (e.g., *IL-10*, *IL1B*, *COX-2*); and pathways related to insulin resistance, an established risk factor for colorectal cancer (e.g., *CAPN10*, *ADIPOQ*, *FABP2*).

Conclusions

Despite the plethora of meat and colorectal cancer research articles, review papers, meta-analyses, and reports—or perhaps as evidenced by their existence—the relationship between red and processed meat and colorectal cancer appears to still be the subject of intense scientific debate [49], with some saying that the very complex evidence and data in total do not clearly point in one direction [5••]. We have presented herein some of the concerns and issues regarding the studies of this relationship. With time, it is certain that the mechanism by which red meat and/or processed meat may cause cancer will be elucidated. At that point, exploration will be needed to determine if the mechanism can be overcome, either through changes in food processing or animal feeding. In addition, more studies are needed that specifically assess the impact of white meat, including the effects of substituting white meat for red meat, and that determine the best tools for more detailed

dietary assessment, which include cooking methods and levels of doneness [14••]. Moreover, the development of a cadre of biomarkers of dietary exposure (i.e., specific to red meat, white meat, processed meat, heated meat) is needed to better elucidate true dietary intake [5••, 14••]. Proposed urinary candidates include total nitrogen and urea, 1- methylhistidines and 3-methylhistidines, carnosine, and trimethylamine oxide, while candidates in both urine and plasma include creatine, creatinine, and carnitine as overall markers of protein intake; trans-fats as markers of ruminant products; and HAA and advanced glycation endpoints as markers of over-heated foods [5••, 53, 54]. Lastly, given the complexity of food and the human diet, a whole-foods approach in study designs seems warranted versus testing of purified compounds. Although a 2014 review of recent epidemiologic studies since 2007 concluded that there was some evidence for a role of red and processed meat in esophageal, liver, kidney, and prostate cancer [14••], it seems ineffective to continue to conduct studies on meat and cancer without first developing the much-needed tools to more accurately assess the relationship.

Compliance with Ethics Guidelines

Conflict of Interest Sabrina P. Trudo and Daniel D. Gallaher declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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