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Effect of White Versus Red Meat on Endogenous N-Nitrosation in the Human Colon and Further Evidence of a Dose Response[1]

Sheila Anne Bingham,[2] Roisin Hughes and Amanda Jane Cross

Medical Research Council, Dunn Human Nutrition Unit, Cambridge, U.K. CB2 2XY

ABSTRACT N-Nitroso compounds are found in the colon and are formed endogenously because amines and amides are produced by bacterial decarboxylation of amino acids in the large gut. They can be N-nitrosated in the presence of a nitrosating agent. To test the hypothesis that increased nitrogenous residues from red meat would increase endogenous N-nitrosation, thus accounting for the epidemiologic association between red meat consumption and colorectal cancer, we fed increased levels of red meat and measured apparent total N-nitroso compounds (ATNCs) in fecal samples in a series of studies of volunteers maintained under controlled conditions. A result of these studies is that we have shown a consistent dose response to red meat consumption. Fiber, in the form of vegetables, bran or resistant starch, does not reduce the level of ATNCs formed, although transit time is reduced and fecal weight are increased. Here we show that the equivalent amount (420–600 g) of meat as white meat has no effect on fecal ATNCs in 12 volunteers ($P = 0.338$). At dosages of 0, 60, 120, 240 and 420 g of red meat/d, mean levels of ATNC output are highly correlated with dose of meat: for concentration ATNC versus dose of meat in g/d, $r = 0.972$, $\beta = 0.252$ ng/g (SE 0.035); for total ATNC output versus dose of meat in g/d, $r = 0.963$, $\beta = 2.605$ μg/d (SE 0.419). The effects of nonmeat protein and of heme on increased N-nitrosation and the genotoxic effects of the ATNCs produced are presently being investigated. J. Nutr. 132: 3522S–3525S, 2002.

KEY WORDS: • *white meat* • *red meat* • *cancer* • *colon* • *N-nitroso compounds*

Up to 80% of colorectal cancer cases have been attributed to diet, suggesting that this cancer, the second most common in Western countries, is a preventable disease (1). Armstrong and Doll (2) attributed much of the international variation in large bowel cancer incidence among countries to dietary differences, especially meat and fat consumption. If meat is associated with increased risk, lower rates for cancer would be expected in vegetarians; in a meta-analysis of five cohorts, non–meat eaters were not at lower risk than meat eaters (3). However, in the largest study of these vegetarians versus meat eaters, meat was associated with increased risk of colorectal cancer (3,4). Two systematic reviews of meat consumption in relation to colorectal cancer incidence were recently published. In 13 prospective studies an increase of 100 g all meat or red meat was associated with a significant 12–17% increased risk of colorectal cancer, and a significant 49% increased risk was found for a daily increase of 25 g processed meat (5). In 34 case-control studies and 14 cohort studies average relative risk was 1.35 [confidence interval [3](CI 1.21–1.51)] for red meat and 1.31 (CI 1.13–1.51) for processed meat (nitrite treated, cured or smoked, including lunch meats) and meat products and there was no significant association with total meat (6). In contrast, white meat consumption has been associated with decreased risk in two prospective studies (7,8).

One explanation for the association between meat and colorectal cancer is the presence of N-nitroso compounds (NOCs) formed endogenously within the colon. NOCs are formed because the amines and amides produced primarily by bacterial decarboxylation of amino acids can be N-nitrosated in the presence of a nitrosating agent (9,10). A number of facultative and anaerobic colonic bacteria can catalyze the formation of NOCs at optimum pH 7.5 (11–13). In the anaerobic large bowel, nitrate is reduced during dissimilatory nitrate metabolism by the colonic flora to nitrite from which nitrosating agents may be formed. Supplements of nitrate have therefore been shown to elevate fecal NOCs (14). N-Nitrosation in the colon has been demonstrated in animals and shown to be dependent on the presence of gut flora (15).

Meat increases the level of nitrogenous residues reaching the colon (16) so that meat might be expected to increase colonic level of NOCs. We previously showed that fecal NOC

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[2] To whom correspondence should be addressed
E-mail: S. Bingham@mrc-dunn.cam.ac.uk.

[3] Abbreviations used: ATNC, apparent total N-nitroso compound; CI, confidence interval; NOC, N-nitroso compound.

TABLE 1

Effect of 420–600 g/d of red meat on fecal N-nitroso compound levels compared with the effects of 60 g/d red meat and 420–600 g/d of white meat in 12 male volunteers

	60 g meat		High red meat		High white meat		
Item	Means	SD	Means	SD	Means	SD	$P^{1,2}$
ATNCs, $\mu g/g$[3]	572	349	2104	1524	759	528	0.014[1] 0.001[2] 0.338[3]
ATNCs, $\mu g/d$	70.4	40.5	249	167	87	55	0.009[1] 0.009[2] 0.408[3]
Fecal weight, *g/d*	131	49	121	32	124	41	0.690[1] 0.390[2] 0.514[3]
Mean transit time, *h*	64	28	66	33	72	41	0.242[1] 0.705[2] 0.299[3]

[1] High white meat vs high red meat.
[2] High red meat vs low red meat.
[3] High white meat vs low meat.
ATNCs, apparent total N-nitroso compounds.

excretion increases during high–red meat diets (17,18) and that a dose response exists (19). We also showed that an increase in fermentable carbohydrate entering the colon in the form of vegetables, bran or resistant starch did not reduce levels of NOCs produced, although these dietary factors will decrease transit time, increase fecal weight and dilute the contents of the large bowel, thus reducing cancer risk (17,18,20).

We have been unable to show an effect of white meat on endogenous N-nitrosation but have only studied two individuals so far (17). Hence the effect of white meat in 12 individuals, at two different levels, is reported here. In addition, the effect of 120 g red meat on endogenous N-nitrosation in nine volunteers is added to previously published data showing a dose-response effect to 60, 240 and 480 g of red meat/d (19).

MATERIALS AND METHODS

Subjects and diets. Eighteen healthy male volunteers (aged 24–74 y) were studied in a metabolic suite. During this time only food that was provided from a standardized menu of normal food was permitted to be eaten. All diets were constant in fat and nonstarch polysaccharide (dietary fiber) and adjusted for the energy needs for each subject with extra bread, low-fat margarine and marmalade. Energy requirements ranged from 10 to 12.5 MJ/d. In protocol 1, seven subjects were studied for three 10-d dietary periods of 60 g red meat, 600 g red meat (as beef and pork) and 600 g white meat (as chicken, turkey or white cod). In protocol 2, five subjects were studied for three 15-d periods. The diets were the same as in protocol 1 except that 420 g red and white meat rather than 600 g was used. In protocol 3, nine subjects were fed 60 and 120 g red meat for 15 d each. To equalize the energy content of the diets, a glucose polymer drink and cream were substituted for meat on the 60- and 120-g diets. All other items of food on each diet of the protocols were the same. Deionized water was given throughout for drinking and used in cooking to keep nitrate intake constant. Diets were randomly assigned by using a crossover design and subjects were their own control. Permission for the studies was given by the Dunn Human Nutrition Unit and Addenbrookes Hospital ethics committees.

Protocols. All fecal samples were collected and stored at −20°C except for those collected on days 8–10 in protocol 1 and 13 and 14 in protocols 2 and 3. These samples were processed to prepare homogenates within 20 min of excretion. Each sample was diluted fourfold with ultrapure deionized water, homogenized in a stomacher (Colworth 3500, Seward) for 20 min and centrifuged at 4500 rpm for 10 min. Each supernatant was filtered, distributed into aliquots and stored at −20°C. Fecal homogenates were analyzed for NOCs and nitrite by the release of nitric oxide after chemical denitrosation via thermal energy analysis (21). NOCs detected by this group-selective method are referred to as apparent total NOCs (ATNCs). All samples collected during the study were weighed and radiographed, and recoveries of radiopaque fecal markers (22) were noted.

RESULTS

In protocol 1, 96% of the total fecal markers administered were recovered (i.e., 4% of markers were present in specimens not collected after the experimental period). In protocol 2, the mean number of markers recovered in the fecal samples by x-ray analysis was 99.0%, and the mean marker recovery from each volunteer ranged from 98.8% to 99.2%. In protocol 3, mean marker recovery was 97.7% (range 99.9–99.6%).

The mean results for all 12 subjects from protocols 1 and 2 are shown in **Table 1**. Both the concentration of ATNCs and the output per day were significantly higher when the diets with 420–600 g red meat were fed compared with the diet with 60 g meat ($P = 0.001$, $P = 0.009$, respectively). However, ATNC levels when the diets with 420–600 g white meat were fed were not significantly different from the low-meat diet ($P = 0.338$ and $P = 0.408$, respectively, for concentration and output per day) and were significantly lower than those obtained with the diet with 420–600 g red meat ($P = 0.014$, $P = 0.009$, respectively, for concentration and output).

Figure 1 shows individual values of fecal ATNC concentration as a percentage change from the 60-g red meat diet. All subjects increased their fecal ATNC concentration on the high–red meat diet, but there was a large individual variation in response. Four subjects exhibited a fourfold increase, whereas eight subjects increased levels by 1–2.5 times. ATNC levels in all subjects decreased on changing from the high–red meat diet to the high–white meat diet to levels found on the low-meat diet except for two subjects consuming the 600-g high–white meat diet in whom levels increased on changing to the white meat diet.

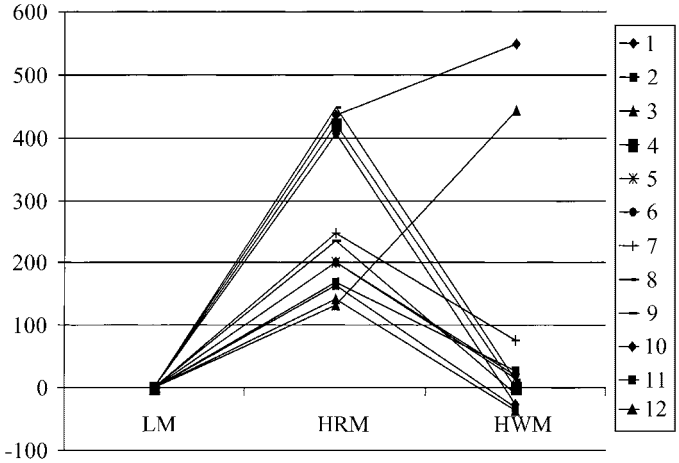

FIGURE 1 Individual changes in fecal ATNC concentration in 12 individuals fed a low-meat (60 g) diet, a high–red meat (420–600 g) diet and a high–white meat (420–600 g) diet. Subjects 1–7 were in group 1 (600 g meat), and subjects 8–12 were in group 2 (420 g meat).

In protocol 3, mean levels of ATNC concentration were 764 $\mu g \cdot kg^{-1}$ (SD 698) on the low-meat diet and 1164 $\mu g \cdot g^{-1}$ (SD 1555; $P = 0.205$) on the high meat diet. ATNC output on the low-meat diet was 77 μg/d (SD 27) compared with 125 μg/d (SD 125) on the high-meat diet ($P = 0.209$). **Figure 2** shows mean values for fecal NOC concentration using combined results from those already published (19) and the results from these nine individuals studied in protocol 3 ($n = 17$ for the 60-g level). Although differences between the 60- and 120-g levels in protocol 3 were not significant, mean levels of fecal ATNCs on the 120-g red meat diet were intermediate between those obtained from the 60-g and 240-g diets (Fig. 2). Mean levels of NOC output were highly correlated with dose of meat: for ng of ATNCs/g versus dose of g of meat/d, $r = 0.972$, $b = 0.252$ (SE 0.035), and for μg of ATNCs/d versus dose of g of meat/d, $r = 0.963$, $b = 2.605$ (SE 0.419).

DISCUSSION

The influence of red meat on fecal ATNC excretion has now been shown in more than 50 healthy male volunteers, all of whom were studied in this study and four previous studies from our laboratory in a metabolic suite where diet could be carefully controlled (17–20). The direction of increase with increasing meat is consistent in nearly all individuals. Furthermore, Figure 2 shows there is a dose response that occurs at normal levels of 120 g of meat/d in addition to the higher levels of 240 and 420 g/d published elsewhere (19). Under these controlled conditions, the dose of red meat was highly predictive of average fecal ATNCs, with R^2 values of 0.97 and 0.96 for concentration and output per day, respectively. At the higher levels of meat consumption, concentrations of ATNCs are of the same order of magnitude as the concentration of tobacco-specific NOCs in cigarette smoke (11,17). We previously showed that fermentable carbohydrate does not change fecal NOC output (17,18,20). In this study, the nonstarch polysaccharide (fiber) contents of the diets were the same and diet had no effect on fecal weight and mean transit time (Table 1).

A high–red meat diet containing 600 g of meat/d provides only 13 μg of preformed ATNCs/d (18). Fecal ATNC levels exceeded this value in all subjects studied so far, showing that fecal ATNC excretion during the study was due to endogenous intestinal formation. This formation is not due to an increase in the amount of nitrosating agents, such as nitrate, because nitrate levels have been kept constant throughout to avoid interference from this factor. An equivalent amount of protein as white meat in this study had no effect; therefore increased endogenous production of nitric oxide from oxidation by nitric oxide synthase of the extra L-arginine present in the high-meat diets is unlikely to account for any increase (23). The 420-g red meat diet would have provided ~7.3 g arginine compared with 1.04 g from the 60-g diet (24).

Despite the consistent response to meat, there is substantial individual variation in the extent of response (Fig. 1). This individual variation remains despite the highly controlled conditions under which studies are carried out. The individual variation may arise from individual differences in gut flora, with high responders harboring high populations of nitric oxide–producing bacteria. Alternatively, individual differences in iron or protein absorption would alter the amount of precursors available for N-nitrosation entering the colon. Iron is required for nitrate reductase activity, which is responsible for bacterially mediated N-nitrosation (11–13).

Although red meat resulted in the expected increase in endogenous N-nitrosation in this study, the same amount of white meat had no effect in 10 of 12 volunteers; therefore mean fecal ATNC levels were not significantly different from those found in a diet with 60 g of meat. In vitro work has shown that the heme proteins myoglobin and hemoglobin in meat react with nitric oxide under anaerobic conditions to form nitrosating agents with the ability to nitrosate phenol (25). Under certain conditions, hemes are known to be nitrosated and act as nitrosating agents (26). The formation of N-nitrosoarginine by heme enzymes under anaerobic conditions was also demonstrated (27). Red meat is a much richer source of heme iron than is white meat; therefore the lack of effect of white meat may be due to a comparative absence of heme. Present studies are investigating the effect of heme on endogenous N-nitrosation (28).

Many classes of NOC have been identified, including nitrosamines, nitrosamides and nitrosoguanidines, and a number of these are known to cause DNA damage after the formation of alkylating agents during NOC metabolism. Methylnitrosourea was shown to induce G→A transitions at codons 12

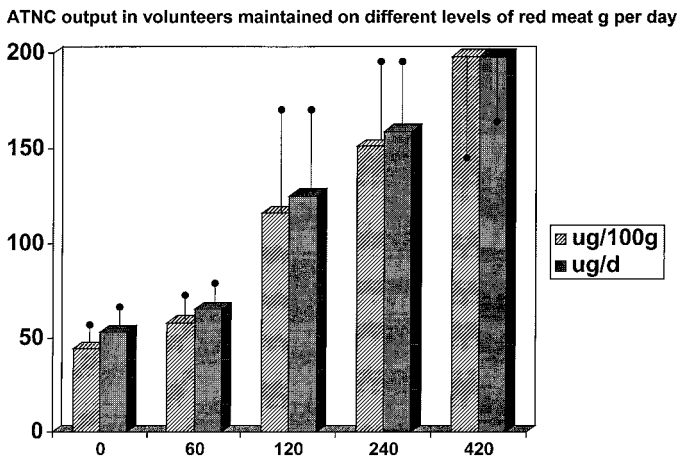

FIGURE 2 Dose response to 0, 60, 240 and 420 g of meat/d and to 120 g of meat/d (from reference 19 and this study). Eight subjects were studied at the 0-, 240- and 420-g level, 9 at the 120-g level and 17 at the 60-g level. Mean and SEM bars are shown.

and 13 in rat colon tumors and is used to induce colon cancer in rat models (29). Endogenous N-nitrosation may thus be the mechanism behind the increased risk of colorectal cancer from red meat, but further work is required to establish the genotoxicity and carcinogenicity of these compounds present in the colon, especially when large amounts of red meat are consumed.

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