Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer?


1 Dunn Clinical Nutrition Centre, Medical Research Council, Hills Road, Cambridge CB2 2DH, UK; 2 International Agency for Research on Cancer, 150, Cours Albert Thomas, 69372, Lyon Cedex 08, France; 3 Pollock and Pool Ltd, Ladbroke Close, Reading RG5 4DX, UK and 4 Nestec Ltd Research Centre, Vers-Chez-les-Blanc, PO Box 44, CH-100 Lausanne 26, Switzerland

To whom correspondence should be addressed

Introduction
Epidemiological studies suggest that the high rates of colorectal cancer in developed countries are potentially preventable by dietary means. National incidence rates for colon cancer are strongly correlated \((r = 0.85)\) with average consumption levels of meat in 23 countries (1). In some prospective studies individuals consuming higher amounts of red or processed meat, but not white meat or fish, experience a greater risk of developing colon cancer (2,3). Vegetarians are known to be at low risk of cancer, including cancer of the large bowel, but it is not clear which aspects of vegetarianism are protective (4,5). Vegetables, starch and non-starch polysaccharides (NSP*), increased stool weight and reduced stool pH are also implicated in reduced risks of colon cancer (6–9). However, the effects of diet on genetic changes which are known in sporadic colorectal cancer have not been established.

\(G\rightarrow A\) transitions at the second G of a GG pair at codon 12 or 13 of \(K\)-ras are common in colorectal cancer and are characteristic effects of alkylating agents such as \(N\)-nitroso compounds (NOC). We studied the effect of red meat consumption on faecal NOC levels in eight male volunteers who consumed diets low or high in meat (60 or 660 g/day), as beef, lamb or pork, whilst living in a metabolic suite. Increased intake of red meat induced a significant \((P < 0.024)\) 3-fold increase from 40 ± 7 to an average of 113 ± 25 ug/day NOC, a range of exposure in faeces similar to that from tobacco-specific NOC in cigarette smoke. The diets were isoenergetic and contained equal amounts of fat, but concentrations of heterocyclic amines were low. Faecal excretion of the promotor ammonia was significantly increased to 6.5 ± 1.08 mmol/day. When the high red meat diets were supplemented with 20 g phytate-free wheat bran in six volunteers there was no reduction in NOC levels (mean 138 ± 41 ug/day NOC), but faecal weight increased. Higher starch and non-starch polysaccharide intakes reduced intraluminal cross-linking in microcapsules \((r = -0.77)\) and reduced faecal pH \((r = -0.64)\). In two volunteers there was no effect of 600 g white meat and fish on faecal NOC (mean low white meat diet 68 ± 10 ug/day, high white meat diet 56 ± 6 ug/day) nor on faecal nitrate, nitrite and iron. Faecal nitrite levels increased on changing from a white to red meat diet (mean high white meat diet 46 ± 7 mg/day, high red meat diet mean 80 ± 7 mg/day). Increased endogenous production of NOC and precursors from increased red meat, but not white meat and fish, consumption may be relevant to the aetiology of colorectal cancer.

Abbreviations: NSP, non-starch polysaccharides; NOC, \(N\)-nitroso compounds; HAA, heterocyclic amines; LM, low meat; HRM, high red meat; HRMHB, high red meat high bran; HWM, high white meat; PEI, polyethyleneimine; ROM, radio-opaque markers; CPTS, copper phthalocyanine; MTT, mean transit time; IQ, 2-amino-3-methylimidazo(4,5-f)quinoline; PhIP, 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine.

Materials and methods

Dietary protocol
Six male volunteers aged 24–32 years were maintained on constant isoenergetic diets for 9 weeks whilst living in the metabolic suite at the Dunn Clinical
Nutrition Centre, where all food was provided and specimens could be collected. Subjects were randomly assigned to either a low (LM) or high red meat (HRM) diet in the first 3 weeks, followed by cross-over for the next 3 weeks and a high white meat, high butter (HRMHB) diet in the last 3 weeks. The diets were isoenergetic and constant in fat throughout. In a subsequent analysis two further male volunteers were fed a low (LM) white meat diet for 5 days, then transferred to a high white meat (HWM) diet for 14 days, followed by a HRM diet for 4 days. The study was approved by the Dunn Nutrition Unit Ethical Committee in 1989.

**Diet and energy balance**

The LM diet contained 60 g meat/day (Table 1) and the HRM diet 600 g cooked meat, of which 400 g was given as fried beef, lamb or pork steak at the evening meal. The meat was substituted with 20 g roast meat and 10 g butter. “Cooked as in methods. Items altered to obtain the LM diet were: 1.5 bottles Hycal and 60 g double cream included; lunch time meats decreased to 25 and 15 g each (40 g total); supper steaks substituted with 20 g roast meat and 10 g butter. "Cooked as in methods."

### Table I. Menus used for HRM diet (10 MJ)*

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
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<tbody>
<tr>
<td>Breakfast</td>
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<tr>
<td>Unsweetened orange juice 100 g</td>
<td>White bread 100 g</td>
<td>White bread 100 g</td>
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<tr>
<td>Weetabix 20 g</td>
<td>Low fat spread 30 g</td>
<td>Low fat spread 30 g</td>
</tr>
<tr>
<td>White bread 50 g</td>
<td>Chicken 100 g*</td>
<td>Turkey 100 g*</td>
</tr>
<tr>
<td>Low fat spread 10 g</td>
<td>Pork 100 g*</td>
<td>Pork 100 g*</td>
</tr>
<tr>
<td>Marmalade 20 g</td>
<td>Pork chops 2×200 g**b</td>
<td>Mayonnaise 12 g</td>
</tr>
<tr>
<td>Dried skim milk powder 30 g</td>
<td>Bottled corn relish 40 g</td>
<td>Hobnob biscuits 15 g</td>
</tr>
<tr>
<td></td>
<td>Crunch biscuits 20 g</td>
<td>Orange 150 g</td>
</tr>
<tr>
<td></td>
<td>Golden delicious apple 150 g</td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
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<tr>
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</tbody>
</table>

*Items altered to obtain the LM diet were: 1.5 bottles Hycal and 60 g double cream included; lunch time meats decreased to 25 and 15 g each (40 g total); supper steaks substituted with 20 g roast meat and 10 g butter. **Cooked as in methods.
NOC determination, but without prior treatment with sulphamic acid, and deducting the nitrte equivalent of NOC. Samples were collected from each volunteer at day 0 (free diet), days 4 and 5 of the LM diet, days 5, 7, 12 and 14 of the HWM diet and day 4 of the HRM diet. In these samples iron was measured by absorption spectroscopy and nitrte by reduction to NO by TiO2 and measurement of the resulting chemiluminescence by thermal energy analysis.

To determine faecal ammonia in the LM, HRM and HRMHB periods of the first study weighed amounts of ~50 g fresh faecal specimen were stomached with an approximately three times weighed amount of 0.1 M HCl for 60 min. Two 20 ml aliquots were centrifuged for 30 min at 3000 rpm and the clear supernatant was removed and stored at -20°C prior to analysis. Ammonia concentration was determined colorimetrically (32).

Faecal and colonic pH
Faecal and colonic pH were determined by radiotelemetry on days 10-13 and days 16-19 of each LM, HRM and HRMHB dietary period (33). The telemetry pills were given after overnight fasting with deionized water at 7 a.m. on days 10 and 16. Subjects took their usual breakfast after the pill left the stomach and monitoring continued until the pill was passed. Faecal pH was taken as the mean of readings at 1, 2 and 3 h after leaving the duodenum. Faecal pH was recorded whilst the pill remained in the faecal specimen until its removal. All telemetry pills were checked for their accuracy before use and after their removal in pH 7 buffer. Only those observations from pills recording pH 6.5-7.5 were used.

Cross-linking and label loss in microcapsules
Cross-linking and label loss was assessed by the semipermeable microcapsule technique (34). Each individual was given 3 ml of an ethanol solution of 14C-labelled (15 kBu) polyethyleneimine (PEI) microcapsules in gelatin-coated enteric capsules by mouth on day 10 of the LM, HRM and HRMHB dietary periods as described previously (35). Fifty radio-opaque markers (ROM), also in gelatin-coated enteric capsules were given at the same time to check that the microcapsules had been released from the enteric coating. On day 16 of the dietary periods 3 ml of microcapsules containing copper phthalocyanine (CPTS) for HAA trapping (36) were given with the evening meal, between the main and dessert courses. PEI-containing microcapsules were removed magnetically from faecal collections over days 10-15 of each dietary period and those containing CPTS from specimens collected over days 16-21. The recovered microcapsules were stored in ethanol before analysis for cross-linking as previously described (37).

Urine nitrate excretion
Twenty four hour urine nitrate excretion was measured by anion exchange chromatography (AS4 guard and column, Dionex) in 24 h urine samples collected on days 10-16 of the LM, HRM and HRMHB dietary periods (24). The 24 h urine collection bottles were double washed with deionized water and contained 10 g NaOH pellets as preservative on days 13-16. Collections on days 16-21. The recovered microcapsules were stored in copper phthalocyanine (CPTS) for HAA trapping (36) were given with the evening meal, between the main and dessert courses. PEI-containing microcapsules were removed magnetically from faecal collections over days 10-15 of each dietary period and those containing CPTS from specimens collected over days 16-21. The recovered microcapsules were stored in ethanol before analysis for cross-linking as previously described (37).

Plasma urea
A fasting blood sample was obtained on days 10 and 16 of the red meat and bran study protocols for plasma urea estimation by hydrolysis with urease to ammonia (Sigma Kit no. 640)

Mean transit time and faecal weight
Faecal weight and mean transit time (MTT) were measured continuously throughout (39). Except where otherwise stated, the subjects took 10 ROM with each meal three times daily. A diary was kept throughout the times of the markers were taken and of faecal collections. All specimens were collected in plastic bags using a frame fitted to the toilet and stored at -20°C prior to X-ray determination of marker content and microcapsule removal. The marker content of each stool and the time the markers were taken was used to calculate MTT and to marker correct faecal weight (39)

Results
In the eight volunteers from both protocols who received the LM and HRM diets faecal NOC was 40 ± 7 µg/day with the LM diet. Analysis of variance showed significant dietary effects (P = 0.031) and there was a significant (P < 0.024) increase by paired t-test to 113 ± 25 µg on the HRM diet (Table II). Figure 1a shows the individual values, with those from the two subjects (7 and 8) included from the second protocol indicated. Levels increased in seven of the eight subjects on changing from the LM to HRM diet. In six volunteers in the first investigation who received the HRMHB diet the mean NOC level on the HRMHB diet was 40 ± 7 µg/day, which was also significantly greater than on the LM diet. Analysis of variance showed significant dietary effects (P = 0.004) and there was a significant decrease in three and decreased in three on changing from the LM to HRM diet. Figure 1b shows the individual values. Levels increased in three and decreased in three on changing from the HRM to the HRMHB diet.

In six volunteers analysis of variance showed significant dietary effects on plasma urea levels (P = 0.001) and 24 h

| n | 8 | All other analyses n = 6. |
Fig. 1. Total faecal NOC output (μg/day) (a) on low red meat (LRM) and HRM diets in eight volunteers with values for two subjects studied in the second protocol indicated and (b) on HRM and HRMHB diets in six volunteers.

Fig. 2. Daily starch (and NSP) intake (g/day), mainly from bread, in relation to (a) faecal weight (g/day) and (b) faecal pH in six volunteers fed three different diets.

Table III. Significant (P < 0.05) Pearson correlation coefficients between continuous variables

<table>
<thead>
<tr>
<th>Faecal weight versus</th>
<th>MTT</th>
<th>-0.644</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-linking index</td>
<td>-0.536</td>
<td></td>
</tr>
<tr>
<td>Starch intake</td>
<td>0.624</td>
<td></td>
</tr>
<tr>
<td>Faecal pH versus</td>
<td>Starch intake</td>
<td>-0.773</td>
</tr>
<tr>
<td></td>
<td>Starch intake</td>
<td>-0.642</td>
</tr>
<tr>
<td></td>
<td>Caecal pH</td>
<td>0.619</td>
</tr>
<tr>
<td>Faecal ammonia versus</td>
<td>Plasma urea</td>
<td>0.688</td>
</tr>
</tbody>
</table>

urine creatinine (P = 0.045). Table II shows that in response to the HRM diet plasma urea increased significantly (P = 0.001), with no change on increasing bran intake. Plasma creatinine did not increase with the HRM diet, although there was a significant (P = 0.005) increase in urinary creatinine output. There were no significant dietary effects on acetylation index, oxidation or 24 h urine nitrate excretion by analysis of variance (Table II).

Analysis of variance showed significant (P = 0.014) dietary effects on faecal ammonia, which increased from 2.72 to 6.50 mmol/L supernatant with the HRM diet. There was a further (non-significant) increase with the HRMHB diet (Table II). There were no dietary effects on MTT or faecal or caecal pH with any of the dietary protocols by analysis of variance, although the HRMHB diet significantly increased faecal weight compared with the HRM diet (P = 0.003). Table III shows that MTT was inversely related to faecal weight (r = -0.644) and faecal pH was related to caecal pH (r = 0.619). Faecal ammonia was related to plasma urea (r = 0.688).

There was no change in cross-linking index in microcapsules with any of the dietary protocols (Table II). However, cross-linking was inversely related to faecal weight (Table III). The use of bread as a supplement to maintain the energy balance in individuals created a continuous dietary variable for starch (and a small amount of NSP) which was inversely related to cross-linking in the microcapsules (r = -0.773) and faecal pH (r = -0.642) and positively correlated with faecal weight (r = 0.624) (Table III). Figure 2a and b shows the positive association between starch and faecal weight and the inverse association with faecal pH. Figure 3a and b shows the inverse
relationships between cross-linking index and starch and cross-linking and faecal weight.

Table IV shows levels of HAA in duplicate samples of the LM and HRM diets. Levels of all HAA measured were higher in the high meat diets, although these differences failed to reach significance. The co-mutagens norharman and harman were found in greatest amounts, followed by 2-amino-3-methylimidazo(4,5-f)quinoline (IQ). There were no detectable levels of 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) present. No HAA were found to be present in the extracts of urine nor in microcapsules recovered from faeces.

Mean NOC levels in the two subjects fed the LM diet over 5 days were 61 ± 10 μg/day, similar to levels found whilst they had been eating a free diet (68 ± 10 μg/day). Over 15 days there was no change with the HWM diet (56 ± 6 μg/day, P > 0.05), but a significant (P < 0.05) increase to 100 ± 9 μg/day after 4 days on the HRM diet. Faecal NOC and nitrite levels were correlated (r = 0.76) and mean levels of nitrite in response to red or white meat showed a similar pattern to those of NOC (Figure 4). Mean levels of nitrite were 59 ± 39, 54 ± 16, 46 ± 7 and 80 ± 7 mg/day in the free, LM, HWM and HRM dietary periods respectively, P < 0.05 HRM versus HWM.

Faecal nitrate levels were low and not significantly different throughout the dietary periods. Mean levels were 0.17 ± 0.33, 0.26 ± 0.13, 0.31 ± 0.11 and 0.21 ± 0.03 mg/day in the free, LM, HWM and HRM dietary periods respectively. When both subjects were considered together there was no significant
correlation between faecal nitrate and nitrite levels \( (r = -0.05) \) nor with faecal NOC \( (r = -0.15) \). Faecal iron on the LM diet was 7.0 ± 0.65 mg/day and 6.7 ± 1.3 mg/day on the HWM diet. The increase to 11.0 ± 5.2 mg/day on the HRM diet was not significant.

Faecal iron levels were significantly higher throughout the study period in volunteer 7 (9.1 ± 1.29 mg/day) compared with volunteer 8 (4.5 ± 0.9 mg/day, \( P < 0.001 \)). Figure 5 shows the trends with time in faecal iron in the separate individuals. Faecal iron levels were correlated with faecal NOC in subject 7 (\( r = 0.714 \)) and with nitrite (\( r = 0.769 \)), but less so in subject 8 (NOC, \( r = 0.442 \); nitrite, \( r = 0.49 \)). In subject 8 there were inverse associations between faecal nitrate and NOC (\( r = -0.53 \)) and between faecal iron and nitrate (\( r = -0.51 \)), but positive associations (\( r = 0.54 \) and 0.20) in subject 8.

**Discussion**

This is the first demonstration in humans that intestinal \( N \)-nitrosation is raised when red meat intake is increased. About a 4-fold increase was shown, with seven of the eight individuals showing an increase with red meat (Figure 1a). The average levels of 113–138 μg/day NOC found on the HRM diets were comparable with other sources of NOC, e.g. tobacco smoke aerosol levels (40). Approximately 30 μg/day of tobacco-specific carcinogenic NOC are obtained from smoking 40 cigarettes/day and the lifetime exposure to faecal NOC, at 2.2 mmol/kg body wt/day, is of the same order as the lowest dose of NOC found to be tumourigenic in rodents (41). This effect of red meat has been confirmed in our more recent study in which a 7-fold increase in faecal NOC levels from 281 ± 63 to 1940 ± 1330 ng/g faeces was found in eight volunteers fed the same amounts of red meat (42). The present report also shows that, in contrast to the effect of red meat, no effect on faecal NOC levels was found when white meat and fish intake was increased to the same level (600 g/day).

**Nature and production of NOC**

The alkaline preservation technique, used with samples from the first six subjects to minimize artefactual formation of NOC, would have destroyed nitrosamides. Subsequent extraction and in vitro treatment of samples homogenized with water instead of alkali have not identified considerable amounts of nitrosamides or nitrosated guanidines, but have shown that both acidic and basic nitrosamines are present. We are presently carrying out further work to characterize the faecal NOC produced, but the presence of carcinogenic or mutagenic NOC cannot as yet be defined. There are conflicting reports on the effects of high meat diets in modulating promotion of colon carcinogenesis in animal models (43,44), but NOC would be important in initiation rather than promotion.

The origin of NOC in faeces is likely to be endogenous. In rats fed diets containing undetectable levels of NOC synthesis was shown to be possible in the large intestine (11) and a number of facultative and anaerobic colonic bacteria are able to catalyse their formation (45). The differential effects of red and white meat in increasing levels could arise from differential effects in the digestion of protein and, hence, the availability of nitrogenous substrates and amines for nitrosation in the colon. However, this was not amenable to study in the present report.

Dissimilatory nitrate metabolism within the colon and, hence, nitrite formation is likely to be important. Nitrate originating from food and drink that reaches the colon is reduced to nitrite and faecal NOC levels increased from an average of 8 μg/100 g faeces on a low nitrate diet to 30 μg/100 g faeces with a supplement of 300 mg nitrate (11). The levels of NOC found with the nitrate supplement were similar to those established here with the LM diets and of the same order as those found in a rural African population (636 ± 148 μg/kg faeces homogenized in NaOH and 573 ± 165 μg/kg homogenized in water) (31).

Faecal nitrite and \( O- \) and \( S- \)nitrosating agents were increased with increased red meat, as evidenced by the significant increase from 46 ± 7 to 80 ± 7 mg/day in faecal nitrite levels obtained when replacing 600 g white meat and fish with 600 g red meat (Figure 4). However, there was no effect on faecal nitrite on changing from a low (60 g) to high (600 g) white meat and fish diet. This suggests that the increase in faecal NOC and nitrosating agents is brought about by a specific effect of red meat not seen with white meat. A major difference between red and white meat is in their content of iron, which is poorly absorbed from the small intestine. However, there was individual variation and an effect of red meat consumption on faecal iron content was only evident in subject 7 (Figure 5). In this subject there were highly significant correlations with faecal iron and both nitrite (\( r = 0.769 \)) and NOC (\( r = 0.714 \)).

Could iron have a role in the production of NO within the colon? Iron is a catalyst for NOC formation and iron and molybdenum are integral components of nitrate reductase and are essential for enzyme activity (46). A previous study in F344 rats maintained with human faecal flora in their intestine and fed human diets showed a 3-fold increase in faecal nitrite reductase with 3-fold increased red meat consumption (47). Faecal nitrate reductase may be a key step in determining the levels of production of NOC and nitrosating agents, such as nitrite from nitrate, and hence in total NOC levels, but we were unable to measure faecal nitrate reductase in the present work. When subjects 7 and 8 were considered together we were also unable to show a significant correlation between faecal nitrite and nitrate (\( r = -0.05 \)) and that there were significant differences in faecal nitrite with the different diets. This and the fact that faecal nitrate levels were low throughout might suggest that iron-dependent activity of faecal nitrate reductase was not the rate limiting step in the conversion of nitrate to nitrite in the colon. However, there were individual differences in faecal iron and nitrite excretion and therefore perhaps in faecal nitrate reductase levels.

Intakes of nitrate and nitrite were constant and low throughout the studies reported here and, hence, the increase in faecal nitrite and other nitrosating agent levels could not have arisen through increased intake. However, NO does not arise from dietary sources of nitrate alone. Increased endogenous production of nitrate via NO synthase is possible from increased dietary arginine levels, from the substantial increase in dietary protein with either red or white meat. The NO would be produced in the epithelium and possibly lead to a localized production of NOC in the adjacent lumen. Studies in animals have shown evidence of increased endogenous nitrosation and increased urinary nitrate with high protein diets (17,18). We found no increase in urine nitrate with the HRM diet and, also in humans, Castillo et al. (48) found no evidence of increased urinary nitrate production on a high versus low arginine diet, but did not measure faecal NOC or nitrite levels. It is possible that increased colonic iron levels may be related to colonic
NO synthase activity and, hence, the increase in nitrite and NOC with red but not white meat, but we have no evidence to demonstrate this at present.

We were unable to show that bran inhibited NOC formation (Table II). There is a possibility that normal bran, which contains substantial amounts of phytate, might have inhibited NOC formation because it inhibits faecal nitrate reductase (49). A role for phytate in protecting against colorectal cancer due to its ability to chelate iron has been proposed (50) and chemopreventive effects of phytate have been shown (51). The original hypothesis related to the formation of reactive oxygen species, but subsequently it has been shown that increased iron did not increase lipid peroxidation products in a rodent colon cancer model (52).

Effects of diet on MTT, ammonia, faecal weight and cross-linking

Despite the inability of the bran used here to modulate faecal NOC levels, faecal weight was increased and, hence, the contents of the lumen diluted. MTT is inversely related to faecal weight (Table III). The net result would have been less contact between NOC arising from the HRM and the colonic mucosa with the HRMB diet. The high meat diet elevated blood urea levels and increased faecal ammonia concentration. The effect of high meat diets in increasing faecal ammonia concentration has been shown before (53). Ammonia in drinking water enhances epithelial cell proliferation in the gastric mucosa and promotes N-methyl-N'-nitro-N-nitrosoguanidine-induced adenocarcinomas in rodents (54,55). Visek (56) implicated elevated faecal ammonia levels in large bowel carcinogenesis. Although NSP can reduce faecal ammonia levels due to a high protein diet via increased fermentation by bacterial flora in the large intestine (53), there was no effect with the bran used in this study. The bran was chosen because it had been treated to be free of starch, nitrate and phytate, but these procedures also render this bran largely unfermentable (57).

Bread intake varied from subject to subject, due to the need to maintain energy balance throughout the study. White bread contains ~1% resistant starch and 1.5% NSP, which reaches the large intestine and is fermented. This should lead to any available nitrogen, as ammonia, being incorporated into bacterial cell walls. The inverse relation between starch intake and faecal ammonia did not reach statistical significance ($r = -0.468$; data not shown), but the increase in biomass produced during fermentation increased faecal weight and there were strong correlations between dietary starch and NSP and faecal weight (Figure 2a).

Short chain fatty acids are also produced during fermentation and the effects of the consequent reduction in stool pH has been discussed in relation to carcinogenesis in the colon (8). In this study there was no effect of unfermentable bran on caecal and faecal pH (Table II), but faecal pH was reduced in response to an increase in starch and NSP from bread ($r = -0.642$, Figure 2b). Fermentation is most rapid in the caecum and, as has been demonstrated elsewhere, caecal pH was lower than faecal pH (33). Caecal and faecal pH were individually correlated ($r = 0.619$; Table III), but caecal pH was not significantly related to intake of starch and NSP from bread ($r = -0.205$; data not shown).

Cross-linking in microcapsules was not increased by high protein diets (Table II), but was significantly reduced in relation to starch intake ($r = -0.773$) and faecal weight ($r = -0.644$) (Figure 3a and b). Cross-linking is indicative of the presence of bi-functional alkylating agents within the colonic lumen (37) and reduced cross-linking with increased starch consumption and, therefore, increased faecal weight is likely to be associated with altered metabolism or dilution of these agents due to increased biomass within the lumen. The effect of reduced cross-linking with increased faecal weight has been shown previously in humans (35).

HAA and phenotyping

The amounts of meat consumed on the high meat diet were within the normal range of day-to-day variation, but contained relatively low levels of HAA. Although little or no PhIP was detected in the duplicate diets analysed, probably because higher cooking temperatures are required (58), levels of HAA were (not significantly) greater on the high protein diets. Microcapsules have previously been shown to trap HAA (36), but no HAA could be detected in extracts from CPTS microcapsules in the present study of humans. No obvious evidence of the typical mutations in ras or p53 have been shown in colon cancers induced in rats by PhIP or IQ (59). When meat is cooked in a conventional manner the finding of elevated faecal NOC may be more consistent with known mutational effects in colon cancer. NOC rather than HAA may therefore be the important factor relating increased meat consumption to colon cancer risk.

We investigated phenotypic changes because HAA and certain N-nitrosamines are activated by P450 enzymes present in the liver and small intestinal mucosa. CYP1A2, which $N$-oxidizes aromatic amines, also catalyses the demethylation of caffeine and, using caffeine as a surrogate to phenotype individuals as fast or slow oxidizers, patients with large bowel cancer have been shown to be faster oxidizers and acetylators than healthy matched controls (21). However, as a consequence of increasing meat intake, protein intake was also altered in this study and there is extensive literature showing a general reduction in P450 enzyme system activity when protein intake is reduced in animals, probably because protein synthesis and liver cell proliferation are retarded (60). These findings also apply to humans, since low protein diets decrease antipyrine and theophylline clearance (61). Changes may occur relatively rapidly, within 1–2 weeks of a change in protein intake in rats (62). Phase II enzyme activity may also be reduced, so that the net result may be an increase or decrease in toxicity of xenobiotics in protein-deficient animals (60). Although diet may therefore affect phenotypic studies of cases and controls in large bowel cancer, we were unable to establish significant dietary effects on acetylation or oxidation in the present study using the caffeine test.

Conclusion

Several changes in intraluminal metabolism in the colon that are related to risk of colon cancer were brought about by the changes in diet in this study. Our present finding of evidence of increased faecal production of NOC and nitrite when red meat consumption is increased is in line with the suggestion that meat may enhance endogenous faecal nitrillation via elevated colonic amine levels and faecal nitrite (62). The increase in endogenous NOC production in the colon from red meat is rapid, occurring within days of a change in diet, and has been now confirmed in three of our study protocols, here and elsewhere (42). The lack of effect of white meat and fish is unexpected, but may relate to faecal iron levels and epidemiological findings that red and processed meat are
associated with increased risk of colon cancer, whereas chicken and fish are associated with decreased risk (2,3).

Acknowledgements

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References


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