

Research Paper

Resistant Starch Prevents Colonic DNA Damage Induced by High Dietary Cooked Red Meat or Casein in Rats

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KEY WORDS

resistant starch, red meat, casein, colon, genotoxicity

ABBREVIATIONS

RS resistant starch
HAS high amylose maize starch
SCFA short chain fatty acid

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ABSTRACT

In a previous study we have shown that high levels of dietary protein (as casein) result in increased levels of colonic DNA damage, measured by the comet assay, and thinning of the colonic mucus layer in rats when dietary resistant starch (RS) is negligible. Feeding RS abolishes these effects. This study aimed to establish whether a diet high in protein as cooked red meat would have similar effects and whether RS was protective. Rats were fed a diet containing 15% or 25% casein or 25% cooked lean red beef, each with or without the addition of 48% high amylose maize starch (a rich source of RS) for four weeks. As expected, high dietary casein caused a 2-fold increase in colonic DNA damage compared with a low casein diet and reduced the thickness of the colonic mucus layer by 41%. High levels of cooked meat caused 26% greater DNA damage than the high casein diet but reduced mucus thickness to a similar degree to casein. Addition of RS to the diet abolished the increase in DNA damage and the loss of colonic mucus thickness induced by either high protein diet. Cecal and fecal short chain fatty acid pools were also increased by inclusion of RS in the diet. Because DNA damage is an early step in the initiation of cancer, these findings suggest that increased DNA damage due to high dietary protein as cooked red meat or casein could increase colorectal cancer risk but inclusion of resistant starch in the diet could significantly reduce that risk.

INTRODUCTION

Colorectal cancer is a socio-economically important disease in affluent societies and epidemiological data suggest that dietary composition is a major factor in its etiology. Earlier reports suggested that high intake of red or processed meats could be a risk factor.¹⁻³ Three large population studies⁴⁻⁶ have recently confirmed those earlier reports. In one of these studies the dietary habits of about 150,000 mature adults were followed over almost 20 years and it was found that prolonged high intakes of red or processed meat were associated with elevated risks of colorectal cancer.⁴ In the largest study, close to 500,000 people were followed over an average period of 4.8 years and again positive associations were found between colorectal cancer incidence and red and processed meat (but not poultry) consumption.⁵ They also found a negative association with fish intake and dietary fibre intake was also protective. A study of over 60,000 Swedish women, followed over an average of 13.9 years, also found a positive association between red meat consumption and development of colon cancer.⁶ The mechanism of this increased colorectal cancer risk is uncertain but may relate to the release of genotoxic agents in the colon.⁷ In contrast, several studies have shown that intake of fruits, vegetables and grains is linked to reduction in risk of colorectal cancer.⁷⁻⁹ It is increasingly evident that in addition to a range of phytochemicals found in these foods, nonstarch polysaccharides (NSP, dietary fibre) and resistant starch (RS) components contribute to their protective effect.¹⁰ RS is the fraction of dietary starch that enters the large bowel undigested.¹¹ RS is known to increase colonic fermentation and studies in animals¹² and humans¹³ have shown increased fecal bulk, lowered fecal pH and increased short chain fatty acid (SCFA) concentrations. Butyrate, a major SCFA, is an energy source for colonocytes and appears to provide protection against activities associated with carcinogenesis.^{14,15} Furthermore, epidemiological studies suggest that risk of colon cancer is attenuated by greater RS consumption,¹⁶ possibly via greater butyrate production.

A previous study in our laboratory, showed that consumption of high levels of protein as casein (25%) caused increased colonic DNA damage, as measured by the comet assay, and thinning of the colonic mucus barrier in rats and that these effects were attenuated by inclusion of RS in the diet.¹⁷ Given the gathering information about the potential role of dietary red meat in colon cancer, we have now examined whether dietary cooked red meat

can cause colonic DNA damage and whether RS is effective in reducing such damage. To our knowledge, this would be the first experimental study to examine the interaction between these dietary variables.

MATERIALS AND METHODS

Animals and diets. Adult, male Sprague-Dawley rats ($n = 48$) weighing approximately 200 g were obtained from the Animal Resource Centre, Murdoch University, Perth, Western Australia. Rats were housed in wire-bottomed cages in a room of controlled heating and lighting (23°C with a 12-h light/dark cycle) and had free access to food and water. They were allocated randomly to six groups ($n = 8$ per group) and fed one of six diets (Table 1) for four weeks.

The diets, based on the AIN-93 diet, contained 15% or 25% casein (low and high casein diets respectively) and 25% red meat (beef round rump boneless steak trimmed of fat; purchased from Central Market Meat (Adelaide, SA), with or without 48% high amylose maize starch (HAS) (*Hi-maize*TM, National Starch and Chemical Company NSW, Australia). Protein quantity for the 25% casein (83% protein) and red meat diets were matched and sunflower oil was used to adjust the overall fat content of diets. The diet without high amylose starch contained highly digestible starch (cornstarch, National Starch and Chemical Company). All diets contained 5% wheat bran as fibre source. Prior to use, the meat was cooked on a hotplate with a temperature of 150°C until lightly browned. The meat was then dried at 45°C for 48 h and milled to provide a product containing 68.5% protein and 22.3% fat.

In the final four days of the experiment rats were placed in metabolic cages to measure fecal output. At the conclusion of the study the rats were anesthetized with 4% halothane/oxygen and gut tissues and digesta were collected for analyses. All procedures involving animals were approved by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Human Nutrition Animal Ethics Committee and the University of Adelaide Animal Ethics Committee.

Comet assay. A 6-cm segment of colon was removed from each rat at a point 3 cm from the most distal end of the colon and colonocytes were isolated immediately for the measurement of DNA strand breaks using the single-cell gel electrophoresis (comet) assay as described previously.¹⁸ The viability of the colonocytes was measured using the trypan blue exclusion method and 100 cells per slide were counted on a hemocytometer. During

electrophoresis under alkaline conditions a DNA “tail” emanates from cells embedded in agarose coated on slides. The length of the tail is related to the extent of DNA fragmentation. Comet tail moment is the product of tail length and the fraction of DNA in the tail and was calculated for 50 cells from each of three slides per rat using Scion Image Beta 4.02 image processing and analysis software (Scion Corp., Maryland, USA) utilising a public domain macro.¹⁹ Apoptotic cells were excluded from the 50 cells as determined by their morphology.

Colonic mucus layer thickness. A 1-cm segment of colon was removed from each rat at a point 2 cm from the most distal end of the colon and cut open along the anti-mesenteric ridge and the mucosal surface was washed gently with 0.15 M aqueous NaCl to remove digesta. The thickness of mucus lining the colon was determined by further cutting the tissue into 1.6 mm lengths, illuminating the tissue segments, capturing numerous images of the mucus layer along each segment, and then measuring the thickness using an image analysis program as described previously.¹⁷ For each animal, ten measurements were taken at different points along 4 tissue segments to give 40 thickness measurements in total.

Short chain fatty acids. The pools of acetate, butyrate, propionate and the total SCFA (including minor SCFA) were determined in the cecal contents and faeces of rats as described previously.²⁰

Statistical analyses. Data are presented as the arithmetic mean \pm SEM for each treatment group. The effects of RS and protein, and their interactions were determined by two-way analysis of variance and differences between treatments were analysed post-hoc by Tukey’s test. These analyses were performed using a SigmaStat statistical software program (SigmaStat 2.0 for Windows, SPSS Inc., Chicago IL USA). A value of $p < 0.05$ was taken as the criterion of significance.

RESULTS

Body weight and intakes of food and water. The mean initial body weight for all groups combined was 207 ± 6 g. Neither body weight gain nor final body weight differed between experimental diets over the 4 wk study. There was an effect of dietary protein treatment ($p < 0.05$), but not HAS treatment, on food intakes (Table 2). Rats fed the 25% red meat diet consumed 18% less than rats fed the 15% casein diets and 12% less than rats fed the 25% casein diets (15.2, 17.5 and 17.1 g (± 0.5) respectively). There was no effect of dietary protein ($P = 0.06$) or HAS on water intakes (Table 2).

Cecal contents and tissue weight, fecal and urine output, intestinal weight and length. The wet weight of cecal contents was over five times

Table 1 **Composition of experimental diets**

| | 0% HAS | | | 48% HAS | | |
|---|------------|------------|-----------------------|------------|------------|----------|
| | 15% Casein | 25% Casein | 25% Meat g/kg Diet | 15% Casein | 25% Casein | 25% Meat |
| Casein | 150 | 250 | 0 | 150 | 250 | 0 |
| Red Meat (cooked lean beef) | 0 | 0 | 300 | 0 | 0 | 300* |
| Cornstarch | 580 | 480 | 480 | 100 | 0 | 0 |
| Hi-Maize TM (resistant starch) | 0 | 0 | 0 | 480 | 480 | 480 |
| Sucrose | 100 | 100 | 100 | 100 | 100 | 100 |
| Sunflower oil | 70 | 70 | 20 | 70 | 70 | 20 |
| Wheat bran | 50 | 50 | 50 | 50 | 50 | 50 |
| L-Cystine | 3 | 3 | 3 | 3 | 3 | 3 |
| Choline bitartrate | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Vitamins (AIN 93) | 10 | 10 | 10 | 10 | 10 | 10 |
| Minerals | 35 | 35 | 35 | 35 | 35 | 35 |
| Tert-butyl hydroquinol | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 |

Diet based on AIN-93 formulation, HAS, high amylose maize starch. *Total protein of 25% meat group was matched with 25% casein group.

Table 2 **Effects of dietary casein, meat and high amylose maize starch levels on daily food and water intakes, fecal and urinary outputs, and cecal and fecal measurements in rats**

| | Dietary Treatment | | | | | | Main Effects (P-values) | | | |
|---------------------------|--------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------------------|-----------|----------------|---------------------|
| | 0% HAS | | | 48% HAS | | | Pooled SEM | RS | Protein | RS × Protein |
| | 15% Casein | 25% Casein | 25% Meat | 15% Casein | 25% Casein | 25% Meat | | | | |
| n | 8 | 8 | 8 | 8 | 8 | 8 | | | | |
| Daily food intake (g) | 17.8 ^a | 16.7 ^a | 15.4 ^b | 18.1 ^a | 17.3 ^a | 15.0 ^b | 0.7 | NS | <0.05 | NS |
| Daily water intake (ml) | 20.3 ^a | 23.4 ^a | 22.0 ^a | 17.1 ^a | 24.7 ^a | 21.5 ^a | 1.6 | NS | 0.062 | NS |
| Cecal Measurements | | | | | | | | | | |
| pH | 7.3 ^a | 7.2 ^a | 6.9 ^a | 5.4 ^b | 5.3 ^b | 5.2 ^b | 0.5 | <0.001 | <0.05 | NS |
| Contents weight (g) | 1.0 ^a | 1.3 ^a | 1.3 ^a | 6.8 ^b | 7.3 ^b | 5.5 ^c | 0.5 | <0.001 | NS | NS |
| Tissue weight (g) | 1.1 ^a | 1.1 ^a | 0.8 ^a | 3.1 ^b | 2.6 ^c | 2.1 ^c | 0.2 | <0.001 | <0.05 | NS |
| Fecal measurements | | | | | | | | | | |
| pH | 6.8 ^a | 7.0 ^a | 7.2 ^a | 5.4 ^b | 5.4 ^b | 5.4 ^b | 0.2 | <0.001 | NS | NS |
| Daily output (g) | 1.7 ^a | 1.7 ^a | 1.8 ^a | 3.9 ^b | 5.1 ^c | 5.6 ^c | 0.3 | <0.001 | <0.05 | <0.05 |
| Daily urine output (ml) | 7.5 | 10.5 | 10.3 | 5.0 | 9.9 | 8.4 | 1.2 | NS | NS | NS |

^{a-c}Data were analysed by two-way ANOVA and post-hoc analysis by Tukey's test. Values are presented as the mean (n = 8) and the pooled SEM. Values in a row not sharing the same superscript are significantly different (p < 0.05). HAS, high amylose maize starch. NS, not significant, p > 0.05.

higher in rats fed HAS than in those fed cornstarch (6.5 versus 1.2 g ± 0.3) (Table 2). This was associated with a doubling of cecal tissue weights and a reduction in the pH of cecal contents (Table 2). There was no significant difference in the weight of cecal contents between rats fed the 15% casein, 25% casein and 25% red meat diets without HAS. However, cecal contents were significantly lower in rats fed the 25% red meat plus HAS diet compared with both casein diets in the presence of HAS. Similar dietary effects were observed for cecal tissue weights. The opposite effect occurred for cecal pH, with a lowering of pH for the meat diet relative to the casein diets in the absence of HAS, whereas there was no difference between protein treatments in the presence of HAS.

Fecal output in the rats fed HAS was 2.7 times greater than that of groups fed the cornstarch diets (4.8 versus 1.7 g ± 0.2), and was significantly higher for 25% meat (p < 0.05) but not 25% casein (p = 0.09) diets compared to the 15% casein diets (3.7, 3.4 and 2.7 g (± 0.2) respectively). In addition, rats fed 25% casein and 25% red meat diets showed significantly higher daily fecal output compared with those fed the 15% casein diet. Daily urine output was not affected by diet. The pH of faeces reflected that of cecal pH in that it was lowered significantly by HAS, but not affected by protein treatment.

There were significant increases in the weights of the small intestine and colon, and length of the colon in rats fed HAS compared to those on diets without HAS (all p < 0.001) (Table 3). There was no effect of protein on any of the above measurements. Small intestine length was unaffected by HAS or protein treatment.

Colonic DNA damage. The viability of isolated colonocytes was 91.4 ± 1.6% and did not differ significantly between treatment groups. DNA damage as measured by comet tail moments was significantly affected by dietary protein treatment (p < 0.001), being greater for the 25% meat and casein diets compared to the 15% casein diets (762 ± 43, 639 ± 40 and 377 ± 40 respectively) (Fig. 1). This was particularly evident in the diets without HAS where comet tail moments for the 25% casein (847 ± 98) and meat (1034 ± 86) diets were significantly increased compared to the 15% diet (398 ± 52) (both p < 0.001), and where the 25% meat diet resulted in greater damage than the 25% casein diet (p < 0.05). The damage was almost twice as great (p < 0.001) in diets without HAS compared to those with HAS (760 versus 425 ± 34). The level of DNA damage for 15% casein diets remained similar in the absence and presence of HAS and there were no significant differences between protein diets in the presence of HAS.

Colonic mucus layer thickness. There was a significant effect of protein (p < 0.001) on mucus layer thickness, with 25% casein and meat diets

reducing colonic mucus layer thickness (p < 0.001), by 41% and 43% respectively, compared to 15% casein diets in the absence of HAS (Fig. 2), and by 20% and 10% respectively in the presence of HAS (Fig. 2). The mucus layer tended to be thicker in diets containing HAS (179 versus 202 μm (± 9); p = 0.09).

Cecal and fecal short chain fatty acids. Acetate, butyrate, propionate and total SCFA pools were measured in the caecum and in the faeces at the end of the study and all were significantly increased by inclusion of HAS in the diet (total SCFA: 101 ± 26 versus 493 ± 26 μmol for caecum and 224 ± 38 versus 598 ± 37 μmol for faeces, both p < 0.001) relative to diets without HAS for all protein treatment groups (Table 4). Although there was generally a significant effect of dietary protein treatment on SCFA levels (except for fecal butyrate) there was no consistent pattern of change for each SCFA in response to the various treatments.

DISCUSSION

Population studies suggest that high levels of dietary protein, especially in the form of red meat, increases the risk of colorectal cancer.^{1-8,21} Experimental evidence suggests fecal mutagens are increased with dietary protein, which could account for the relationship by initiating adverse genomic events.^{17,22} The present study confirms and extends a previous report in which we showed that a 25% casein diet increased colonic DNA damage relative to a diet containing 15% casein in rats.¹⁷ In this experiment, substitution of red meat for casein further elevated the colonic DNA damage by 26% over the 25% casein diet. Although fecal water from individuals on a diet high in fat and meat but low in fibre has been shown to have an increased genotoxic potential,²³ this is the first clear demonstration that a diet high in red meat does increase colonic DNA damage in experimental animals. Since genetic damage is a prerequisite for cancer initiation, these data support the epidemiological data implicating dietary protein, especially cooked red meat, as risk factors for this disease. However, we believe that the abolition of high dietary protein-induced DNA damage by the inclusion of HAS in the diet is an important finding. We had previously demonstrated that HAS could attenuate dietary casein-induced colonic DNA damage,¹⁷ but now extend that effect to DNA damage induced by cooked red meat. This supports the epidemiological and experimental

Table 3 **Effects of dietary casein, mea and high amylose maize starch levels on colon and small intestine length and weights**

| | Dietary Treatment | | | | | | Main Effects (p-values) | | | |
|--------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------------|--------|---------|--------------|
| | 0% HAS | | | 48% HAS | | | Pooled SEM | RS | Protein | RS × Protein |
| | 15% Casein | 25% Casein | 25% Meat | 15% Casein | 25% Casein | 25% Meat | | | | |
| n | 8 | 8 | 8 | 8 | 8 | 8 | | | | |
| Colon Measurement | | | | | | | | | | |
| Weight (g) | 1.2 ^a | 1.1 ^a | 0.9 ^a | 1.4 ^b | 0.9 ^a | 1.4 ^b | 0.08 | <0.001 | NS | NS |
| Length (cm) | 16.4 ^a | 17.3 ^a | 17.3 ^a | 19.0 ^b | 17.3 ^a | 18.8 ^b | 0.7 | <0.001 | NS | NS |
| Small Intestine | | | | | | | | | | |
| Weight (g) | 6.9 ^a | 7.7 ^a | 7.6 ^a | 8.5 ^b | 7.6 ^a | 8.9 ^b | 0.3 | <0.001 | <0.05 | NS |
| Length (cm) | 108.3 | 113.9 | 112.8 | 114.9 | 112.8 | 121.5 | 3.7 | NS | NS | NS |

a,b: Data were analysed by 2-way ANOVA and post-hoc analysis by Tukey's test. Values are presented as the mean (n = 8) and the pooled SEM. Values in a row not sharing the same superscript are significantly different (P < 0.05). HAS, high amylose maize starch. NS, not significant, P > 0.05.

data^{10,17} suggesting that foods containing RS are likely to play a role in protecting against colon cancer.

The mechanism of dietary protein-induced colonic DNA damage in the absence of RS may be related to undigested protein reaching the colon. Increased intake of dietary protein would be expected to lower its ileal digestibility and greater entry of protein into the large bowel would result in its enhanced fermentation by the microflora, yielding end products such as phenol, cresol, indoles, amines and ammonia.¹¹ Phenol and cresol are by-products of the metabolism of aromatic amine acids and they are implicated in bladder and bowel cancers.^{24,25} Our previous study showed there was a relationship between increased fecal phenols and para-cresol concentrations and increased DNA damage associated with consumption of high levels of dietary casein in the absence of HAS.¹⁷ In the present study, cooked red meat caused a significant increase in colonic DNA damage compared to the casein diet. Heterocyclic amines and nitrosamines are compounds linked to dietary intake of cooked meats and increased risk of colon cancer,²⁶⁻³⁰ and may be responsible for the greater damage that we have observed. Additionally, haem iron present in red meat, but not casein, is also implicated as a risk factor for colorectal cancer.³¹

Our study has confirmed previous reports that RS enriched diets can significantly change the luminal environment.^{10,20} The rats fed HAS in the present study showed significant increases in digesta weights and SCFA pools (including butyrate) in the caecum and feces, along with lowered pH of the faeces and cecal digesta, and increased weight of the caecum and length of the colon. These significant changes are consistent with increased bacterial fermentation and for a role of SCFA in protection against protein-induced DNA damage. A study by LeLeu et al²⁰ has shown that diets containing 20% or 30% HAS increase the acute apoptotic response to colonic DNA damage induced by a challenge with azoxymethane in rats, and this was associated with increased SCFA, including butyrate, in the large bowel. This suggests that RS can protect against the consequences of DNA damage induced by a carcinogen. Butyrate is known to enhance differentiation, cell-cycle arrest and the apoptotic

Figure 2. Effects of dietary casein, cooked red meat and resistant starch levels on the thickness of the mucus lining the colon. Data are presented as the arithmetic mean ± SEM (n = 8). Values not sharing the same superscript are significantly different (p < 0.05). HAS, high amylose maize starch.

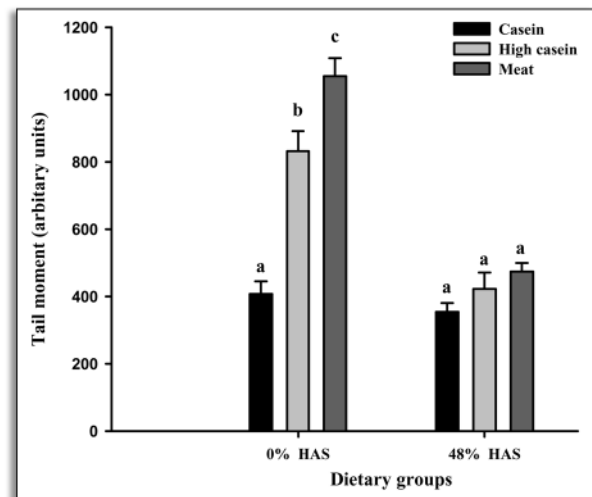


Figure 1. Effects of dietary casein, cooked red meat and resistant starch levels on colonic DNA damage in rats. The comet assay was performed on colonocytes extracted from the colon and the resulting comet tail moments (comet tail length × % DNA in the tail) are presented. Data are presented as the arithmetic mean ± SEM (n = 8). Values not sharing the same superscript are significantly different (p < 0.05). HAS, high amylose maize starch.

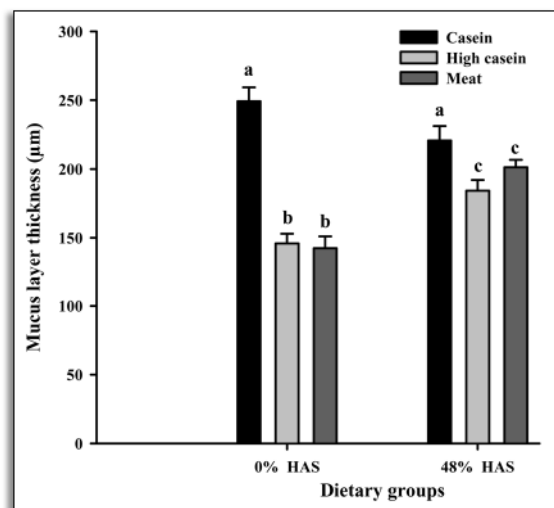


Table 4 **Effects of dietary casein, meat and high amylose maize starch levels on fecal and cecal short chain fatty acid pools (SCFA) in rats**

| | Dietary Treatment | | | | | | Main Effects (p-values) | | | |
|-------------------------|--------------------------|-------------------|------------------|-------------------|-------------------|------------------|--------------------------------|-----------|----------------|---------------------|
| | 0% HAS | | | 48% HAS | | | Pooled SEM | RS | Protein | RS × Protein |
| | 15% Casein | 25% Casein | 25% Meat | 15% Casein | 25% Casein | 25% Meat | | | | |
| n | 8 | 8 | 8 | 8 | 8 | 8 | | | | |
| | | | | μmol | | | | | | |
| Cecal SCFA Pools | | | | | | | | | | |
| Acetate | 66 ^a | 59 ^a | 47 ^a | 432 ^b | 361 ^{bc} | 294 ^c | 39 | <0.001 | <0.05 | NS |
| Propionate | 17 ^a | 18 ^a | 17 ^a | 83 ^{bc} | 107 ^c | 59 ^b | 7 | <0.001 | <0.05 | NS |
| Butyrate | 23 ^a | 19 ^b | 21 ^a | 56 ^c | 54 ^c | 51 ^c | 4 | <0.001 | <0.05 | NS |
| Total SCFA | 111 ^a | 101 ^a | 90 ^a | 584 ^b | 663 ^b | 413 ^c | 45 | <0.001 | <0.05 | NS |
| Fecal SCFA Pools | | | | | | | | | | |
| Acetate | 194 ^a | 157 ^a | 228 ^a | 396 ^b | 620 ^c | 602 ^c | 63 | <0.001 | <0.05 | NS |
| Propionate | 16 ^a | 15 ^a | 25 ^a | 20 ^a | 50 ^b | 29 ^a | 4 | <0.001 | <0.05 | <0.001 |
| Butyrate | 3 ^a | 3 ^a | 4 ^a | 13 ^b | 17 ^b | 12 ^b | 2 | <0.001 | NS | NS |
| Total SCFA | 222 ^a | 182 ^a | 264 ^a | 435 ^b | 705 ^c | 657 ^c | 66 | <0.001 | <0.05 | NS |

^{a-c}Data were analysed by two-way ANOVA and post-hoc analysis by Tukey's test. Values are presented as the mean (n = 8) and the pooled SEM. Values in a row without sharing the same superscript are significantly different (p < 0.05). HAS, high amylose maize starch. NS, not significant, p > 0.05.

response in the colon,³²⁻³⁴ and is widely regarded as a mediator of the beneficial effects of RS in the colon.¹⁰ However, there is some conflicting evidence in the literature regarding the role of butyrate in maintaining bowel health (the butyrate paradox).³⁵ In our present study RS may act to improve colonic health in a number of possible ways. RS may also act to prevent DNA damage, as dietary RS is known to lower fecal concentrations of the toxic by-products of protein metabolism such as ammonia and phenols in humans,³⁶ and RS was also shown to lower fecal phenol concentrations in rats.¹⁷ Dietary complex carbohydrates in general appear to be able to reduce the amount of these harmful products in the large bowel.^{37,38}

We have previously shown that a thinner colonic mucus layer coexists with increased DNA damage induced by high dietary casein when RS is negligible.¹⁷ We now show that high levels of both dietary casein and cooked red meat substantially reduce mucus layer thickness coincidentally with increased colonic DNA damage and that this is reversible with inclusion of HAS in the diet. The mucus barrier and fecal mucinolytic activities are often altered in large bowel diseases.³⁹ Recently, a rodent study showed that mucin-depleted foci in the colon correlate with tumor induction in azoxymethane-treated rats.⁴⁰ Furthermore, using this methodology, Pierre et al.⁴¹ showed that rats fed beef meat had increased numbers of mucin-depleted foci as well as aberrant crypt foci compared with casein. Despite these associations, a role for the mucus barrier in protecting the colon against genotoxic agents and carcinogenesis is yet to be established. A change in mucus thickness or composition may simply be a response to colonic damage. Nevertheless, there is growing evidence that dietary complex carbohydrates have a dynamic effect on colonic mucus. Shiau and Ong⁴² observed an increased fecal mucinase activity in rats fed fibrefree diets and proposed that possible changes in the mucus layer may alter the accessibility of harmful agents to the colonic mucosa. Fibrefree diets have since been shown to decrease both the thickness of this mucus layer and the secretion rate of mucus in rats in vivo.⁴³ Fermentation of complex carbohydrates and subsequent release of SCFA, particularly butyrate, may be a mechanism through which the colonic mucus barrier is maintained. A recent in vitro study using a colonic goblet cell line demonstrated

that genes for mucins were upregulated by butyrate when it is the major energy source of the cell.⁴⁴ This is supported by our current study, as cecal and fecal SCFA pools were increased in association with inclusion of HAS in the diets and in association with greater mucus thickness. However, an earlier study using the same cell line showed conflicting results.⁴⁵

All rats in the present study were fed diets that contained 5% wheat bran, as it is possible that the effects of RS could be sub-optimal in the absence of fibre. Recently, Morita et al.⁴⁶ found that non-purified diets supplemented with RS performed better at generating cecal SCFA than fibrefree diets supplemented with RS in rats. Other work has shown that a mixture of polysaccharides in the diet may be better than any one polysaccharide alone in facilitating fermentation in the large bowel.⁴⁷ This suggests that inclusion of fibre in the diets of our study is likely to have increased the level of SCFA production above that which would have been seen had fibre been absent. This increased SCFA production, combined with the fecal bulking and increased fecal transit time associated with fibre consumption, is expected to have contributed to a reduction in the levels of colonic DNA damage across the treatment groups. Indeed, wheat bran has been shown to increase butyrate levels and reduce the number of aberrant crypt foci in an experimental model of colon cancer.⁴⁸

Morita et al.⁴⁹ showed in their rodent study that metabolism of dietary protein can affect cecal SCFA pools. Inclusion of resistant protein in the diet increased the butyrate pool but lowered succinate. However, our study demonstrated that increased protein in the diet did not affect colonic SCFA pools. Moreover, we showed that there was a significant drop in the total cecal SCFA pool of red meat fed rats compared to both low and high casein fed rats. A reduction in the levels of acetate and propionate led to this drop in total SCFA. Both acetate and propionate can induce apoptosis, but to a lesser extent than butyrate.⁵⁰ The reduced pools of these two major SCFA in meat fed rats may have contributed to the increased colonic DNA damage we observed in these animals.

In summary, this study shows increased dietary protein, as either casein or cooked red beef, particularly in the absence of resistant starch, increases colonic DNA damage in rats and causes thinning of

the colonic mucus layer. These changes are likely to increase the risk of colon cancer. Inclusion of resistant starch in the diet attenuated these changes, possibly as a result of the actions of butyrate, which was increased in the large bowel. Although further investigations are necessary to ascertain whether dietary protein and resistant starch exert similar effects in humans, our data support a role for RS in protecting against colorectal cancer.

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