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REVIEW

Relevance of protein fermentation to gut health

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It is generally accepted that carbohydrate fermentation results in beneficial effects for the host because of the generation of short chain fatty acids, whereas protein fermentation is considered detrimental for the host's health. Protein fermentation mainly occurs in the distal colon, when carbohydrates get depleted and results in the production of potentially toxic metabolites such as ammonia, amines, phenols and sulfides. However, the effectivity of these metabolites has been established mainly in in vitro studies. In addition, some important bowel diseases such as colorectal cancer (CRC) and ulcerative colitis appear most often in the distal colon, which is the primary site of protein fermentation. Finally, epidemiological studies revealed that diets rich in meat are associated with the prevalence of CRC, as is the case in Western society. Importantly, meat intake not only increases fermentation of proteins but also induces increased intake of fat, heme and heterocyclic amines, which may also play a role in the development of CRC. Despite these indications, the relationship between gut health and protein fermentation has not been thoroughly investigated. In this review, the existing evidence about the potential toxicity of protein fermentation from in vitro animal and human studies will be summarized.

Keywords:
CRC / Fecal water genotoxicity / Prebiotic / Probiotic / Protein fermentation

1 Introduction

Anaerobic degradation of undigested or endogenous protein in the colon by the resident microbiota, a process also called putrefaction, is generally presumed to be detrimental for the host's health [1–5]. Protein fermentation results in the production of a wide range of metabolites that are in direct contact with the colonic mucosa and can directly interact with the mucosal cells. Protein fermentation has mainly been implicated in the etiology of colorectal cancer (CRC) but also in ulcerative colitis (UC) and in physiological processes such as aging. Nowadays, the impact of protein fermentation on gut health has become particularly relevant in view of the widespread application of high protein diets for weight loss and body weight management.

Already more than three decades ago, the hypothesis was raised that the fermentation of proteins may contribute to the development of colon cancer [6]. Hill et al. postulated that bacterial metabolism of dietary or endogenous compounds was involved in the etiology of colon cancer and pointed at the bacterial conversion of bile acids into compounds with co-carcinogenic properties [7]. However, the observed association between high meat intake and increased frequency of colon cancer could not be explained by increased bile acid concentrations in the colon as meat intake only has a small effect on fecal bile acid excretion [6]. The fact that protein is a major constituent of meat and that protein fermentation metabolites like ammonia (NH_3), phenolic compounds or tryptophan metabolites were found potentially carcinogenic suggested a possible link between meat intake, protein fermentation and colon cancer.

Additional indications for the detrimental effects of protein fermentation on gut health were derived from the observation that protein fermentation becomes more

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Abbreviations: **BCFA,** branched chain fatty acid; **CD,** Crohn's disease; **CRC,** colorectal cancer; **FOS,** fructooligosaccharides; **IBD,** inflammatory bowel disease; **OF-IN,** oligofructose-enriched inulin; **RS,** resistant starch; **SCFA,** short chain fatty acids; **UC,** ulcerative colitis

dominant in those regions of the colon that are most affected by diseases, i.e. the distal colon. UC starts at the rectum and spreads proximally and about 60% of the large bowel cancers are located in the distal colon or rectum [8]. Chao et al. evaluated the impact of prolonged high meat intake and found that prolonged high consumption of red and processed meat may increase the risk of cancer in the distal portion of the large intestine [9].

In this review, we provide an overview of the process of protein fermentation and summarize the available evidence on the toxicity of protein fermentation from in vitro, animal and human studies. Nevertheless, proteins form an essential part of the human diet and are important for processes like muscle growth, immunity and reproduction. However, these aspects have been extensively reviewed elsewhere [10] and have not been included in this review.

2 Factors affecting protein fermentation

In western diets, on average 15–20% of energy intake is derived from protein intake [11]. The amount of protein entering the colon depends on the protein content of the ingested food and protein digestibility. Digestibility of proteins from animal sources (dairy and animal proteins) exceeds 90% and is generally higher than the digestibility of plant proteins (70–90%). Dairy proteins, whey and casein, appear to be slightly more digestible than meat proteins [12]. Using stable isotope techniques, Evenepoel et al. compared the digestibility of raw and cooked egg protein and showed that the amount of protein entering the colon and the amount of fermentation metabolites retrieved in urine depends on the digestibility of the proteins [13]. Similarly, thermolysation of casein (heating at 180°C for 1 h) significantly decreased the digestibility of the protein and increased the degree of protein fermentation [14]. However, in ileostomy patients, digestibility of different sources of protein (beef and cheese) was similar and ileal nitrogen output was strongly correlated to dietary nitrogen intake ($p < 0.0001$) suggesting that, on a normal mixed diet, it is the amount of protein in the diet rather than its source that determines the amount reaching the colon [2].

3 Products of protein fermentation

Degradation of proteins in the colon starts with hydrolysis of the proteins to smaller peptides and amino acids by bacterial proteases and peptidases that are more active at neutral to alkaline pH. In the proximal colon, pH is more acid due to the production of short chain fatty acids (SCFA) from carbohydrate fermentation. Upon progression to more distal parts of the colon, carbohydrates get depleted, pH increases and protein fermentation becomes more efficient.

Although SCFA are the major end products from carbohydrate fermentation, they are also produced from many amino acids by reductive deamination [15]. SCFA are rapidly absorbed from the colon and are generally considered to be beneficial for the host. Butyrate is the most important energy source for colonocytes and plays a major role in proliferation and differentiation [16]. Additional functions include inhibition of colonic carcinogenesis and inflammation, reduction of oxidative stress and reinforcing the colonic defense barrier [17].

Contrary to SCFA, branched chain fatty acids (BCFA) exclusively originate from fermentation of branched amino acids. Isobutyrate, isovalerate and 2-methylbutyrate are produced from the fermentation of valine, leucine and isoleucine, respectively [18].

NH_3 is produced by bacteria through deamination of amino acids and to a lesser extent through urea hydrolysis catalyzed by bacterial urease activity [15]. Up to 3.5–4.0 g of NH_3 is released every day in the gut [19], resulting in luminal concentrations in humans up to 60 mmol/kg of luminal content [20]. NH_3 can be used by the bacteria for their own metabolism and protein synthesis. Alternatively, it is absorbed by the colonocytes, transformed to urea in the liver and excreted in urine.

Bacterial degradation of aromatic amino acids in the colonic lumen results in the production of phenolic and indolic compounds (Fig. 1). Degradation products from tyrosine include 4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate, 4-hydroxyphenylpropionate and 4-hydroxyphenylacetate as well as phenol, *p*-cresol and 4-ethylphenol. Phenylalanine bacterial metabolism leads to similar derivatives, i.e. phenylpyruvate, phenyllactate, phenylacetate and phenylpropionate. Tryptophan degradation generates indole, 3-methyl indole (skatole), indole acetate and indole propionate. Phenolic compounds are largely absorbed from the colon, detoxified in the colon mucosa and the liver by glucuronide and sulfate conjugation, and finally excreted in urine. More than 90% of urinary phenolic compounds are excreted as *p*-cresol [21].

BCFA, phenols and indoles are not produced by human enzymes and are therefore unique colonic bacterial metabolites. As a consequence, excretion of those metabolites is often considered as a marker to estimate the degree of protein fermentation in the colon [22].

Fermentation of dietary and mucinous sulfate and sulfur amino acids, such as methionine, cystine, cysteine and taurine, by sulfate-reducing bacteria results in the production of hydrogen sulfide (H_2S) [23, 24]. H_2S is an extremely toxic agent (LD_{50} for rodents comparable to cyanide [25]) and luminal H_2S concentrations in the large intestine range between 1.0 and 2.4 mM [26]. The consumption of sulfur amino acids fluctuates with protein intake. A randomized cross-over trial in five healthy men compared fecal sulfide concentration after a 10-day diet ranging from 0 g/d meat (51 g/d protein) to 600 g/d meat (212 g/d protein). Fecal sulfide concentration correlated significantly with dietary protein intake ($p < 0.001$) [27].

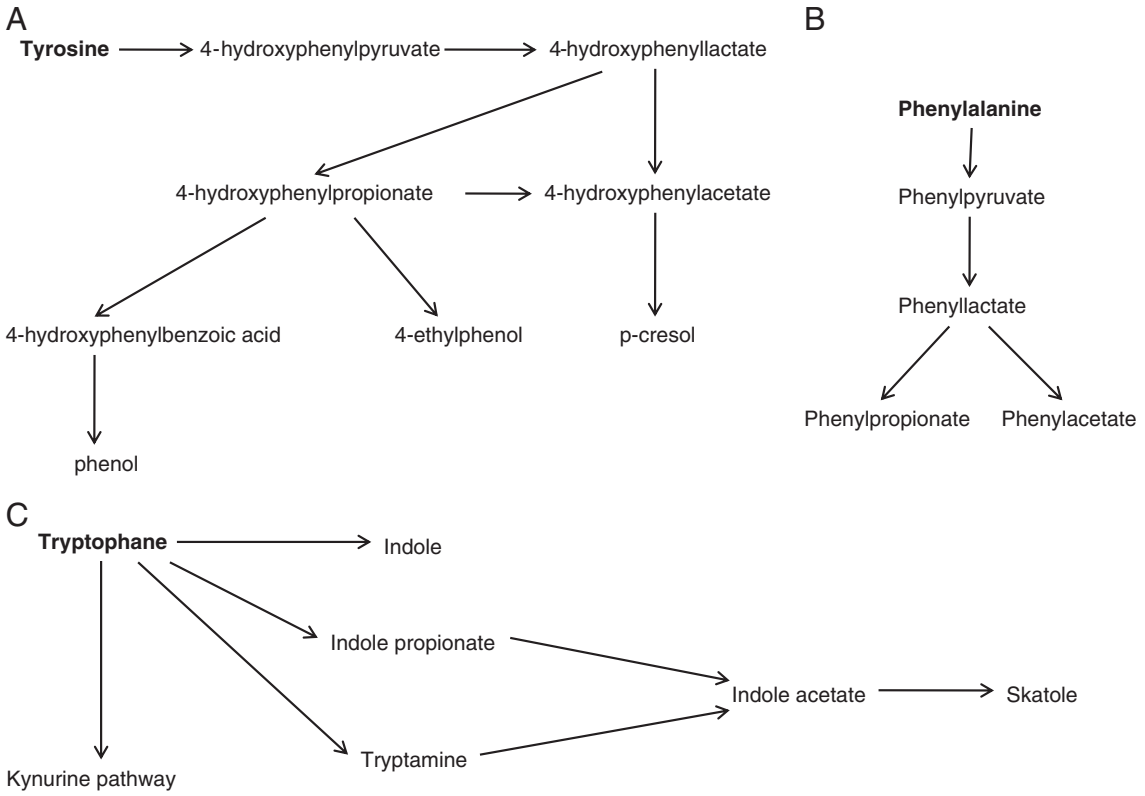

Figure 1. Degradation pathways of the aromatic amino acids tyrosine (A), phenylalanine (B) and tryptophan (C).

Finally, decarboxylation of amino acids results in the appearance of amines in the gut. Luminal amines and polyamines arise from endogenous secretion, from dietary polyamines which have not been absorbed and from release by desquamated cells [15]. Monoamine and diamine oxidases present in the gut mucosa detoxify the amines produced by the gut microbiota. In addition, amines play a role in the formation of *N*-nitrosamines by condensation of a secondary amine with nitrite in an acidic environment or at neutral pH, when catalyzed by bacterial enzymes [28].

4 Toxic potential of protein fermentation

4.1 In vitro studies

The effects of protein fermentation have greatly been derived from in vitro experiments, in which cells, isolated colonic crypts or colon tissue, were exposed to individual potentially harmful metabolites.

4.1.1 Ammonia

Due to bacterial degradation and endogenous nitrogen recycling, the colonic epithelium is constantly exposed to NH_3 [18]. Topping et al. found that pyrimidine synthesis and subsequent incorporation into RNA in gut epithelial cells was stimulated after incubation with ammonia [29]. Incubation of isolated distal rat colons with ammonia (75 mM) stimulated epithelial cell proliferation without changing crypt size [30]. In contrast, exposure of isolated pig colonic crypts for 4 h to NH_4Cl (50 mM) did not alter cell viability as judged from membrane integrity measurements [31].

Bromodeoxyuridine labeling was investigated after incubating human biopsies from the ascending colon with 10 mM ammonium butyrate or equimolar Na-butyrate [32]. Surprisingly, no difference in labeling indexes was observed. It was hypothesized that a toxic effect of ammonium was counteracted by the differentiating effect of butyrate.

4.1.2 *p*-Cresol and phenol

Viability of colonic epithelial cells isolated from human biopsies was decreased after exposure to 1.25 mM phenol, a physiologically relevant concentration, whereas clearly higher phenol concentrations (20 mM) were required to reduce viability of HT-29 cell [33]. Transepithelial resistance of Caco-2 cells was decreased after incubation with NH_3 (10–100 mM), phenol (1–10 mM) and both primary and secondary bile acids (50–250 μM) [34]. McCall et al. confirmed the effect of phenol on cell permeability in

SK-CO15 cells, which are also transformed human intestinal epithelial cells. This effect was dose-dependent and increased with length of exposure [35]. Permeability of endothelial cells was also significantly decreased after exposure to *p*-cresol (10–50 μg/mL) [36]. Thinning or increased permeability of the mucous layer is likely to increase the accessibility of a wide range of agents to the colonic mucosa, including genotoxic agents.

4.1.3 Hydrogen sulfide

The toxic potential of H_2S on colonic cells is well documented. After exposure to sodium hydrogen sulfide (NaHS), proliferation in non-transformed rat intestinal crypt cells (IEC-18-cells) was increased [37]. A series of in vitro experiments by Attene-Ramos et al. revealed that H_2S affects different cellular pathways at concentrations similar to those found in the colon. Sulfide provokes genomic DNA damage in colonic cancer cells (HT-29 cells) at concentrations of 250 μM as evidenced by a modified Comet assay in which DNA repair was inhibited [38]. Similar results were obtained when naked nuclei from Chinese hamster ovary cells were treated with sulfide (1 μM), indicating that no cellular metabolism is required for sulfide to induce genotoxicity. In addition, the number of oxidized bases was increased after treatment with sulfides. Co-incubation with butylhydroxyanisole, a radical scavenger, reduces DNA damage induced by H_2S, suggesting that this damage could be radical-mediated [39]. In non-transformed human intestinal epithelial cells, the expression of genes involved in cell-cycle progression, inflammation and DNA repair response was modulated by sulfide. Expression of the COX-2 gene, which is elevated in most human CRC's, was significantly upregulated [40].

Besides inducing DNA damage, sulfide prevents the oxidation of butyrate in colonocytes. Butyrate oxidation was inhibited after exposure of normal rat colonocytes to sulfide (0–2.5 mM) [41]. Inhibition of butyrate oxidation induces an energy-deficient state ultimately resulting in reduced absorption of sodium, reduced secretion of mucin and a shorter life of the colonocytes [24]. H_2S also inhibits cellular respiration, at least in part by acting as an inhibitor of cytochrome *c* oxidase, which is the final step in the production of adenosine triphosphate [42]. In colonic epithelial cell homogenates, micromolar concentrations (0.5–5 μM) of NaHS inhibited cytochrome *c* oxidase activity [43].

4.2 Animal studies

4.2.1 Effect of protein fermentation metabolites

Perfusion of rat colon with 35 mM ammonium acetate/chloride as compared with a saline solution induced significant histological mucosal damage and loss of mucus [44]. In a rat model with chemically (*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine) induced colon carcinogenesis, intrarectal ammonium acetate infusion during 52 wk resulted in an increase in colonic adenocarcinoma [45].

Several lines of experimental evidence implicate sulfide as a damaging agent in the pathogenesis of UC. In experimental animal models, it is possible to induce a pathological state similar to the one observed in UC using two forms of indigestible sulfates, i.e. dextran sulfate sodium and sulfate-containing carrageenan [15].

In rats treated with NaHS (10–30 mM) or saline via a stoma for 4 (acute) or 90 (chronic) days, butyrate oxidation was reduced in both the acute and chronic experiments [46].

4.2.2 Effect of protein intake

Corpet et al. investigated in rats the association between the production of colonic protein fermentation metabolites and the promotion of colon carcinogenesis [14]. Casein, soy protein and egg white were thermolysed to decrease their digestibility and were added to the diets of rats. Heating of casein for 1 h increased protein fermentation evidenced by fecal ammonia and urinary phenols. However, heating for 2–4 h led to a less marked effect. In contrast, the promotion of colonic aberrant crypt foci was highest upon administration of casein thermolysed for two or more hours. Thermolysis of soy and egg white increased colonic protein fermentation but did not promote the aberrant crypt size. These results do not support the hypothesis that protein fermentation products play an important role in colon cancer promotion.

The most convincing evidence relating protein fermentation to CRC risk is derived from a series of experiments in rats. Those studies investigated the impact of dietary interventions with different sources and amounts of protein on CRC risk.

As compared with a normal protein diet (15% casein), colonocyte genetic damage was significantly increased when rats were fed a high protein diet as casein (25%), soya (25%), white or red meat (25 and 35%), but not whey protein (25%) [47–51]. Red meat induced more genetic damage as compared with white meat [48], which was explained by the fact that red meat contains higher amounts of heme than white meat. Heme stimulates the production of genotoxic endogenous *N*-nitroso compounds in the human gut [52].

Consumption of a high (25%) casein diet was associated with significantly increased levels of fecal *p*-cresol as compared with a 15% casein diet. In addition, *p*-cresol levels correlated significantly with genetic damage ($p < 0.001$) [46]. Also after consumption of a high red and white meat diet, cecal and fecal *p*-cresol levels were significantly increased [49]. Although no correlation was provided with the extent of genetic damage, these results suggest an association between protein fermentation and genetic damage.

Substitution of casein by potato protein, which has a lower digestibility, in the diet of rats significantly increased the urinary excretion of *p*-cresol and intestinal BCFA concentrations and was associated with increased intestinal tumorigenesis [4]. Interestingly, mainly small intestinal tumors increased after administration of potato protein. It was suggested that although harmful fermentation products are produced in the colon, they might act systemically on small intestinal cells.

In addition, the increased DNA damage after high protein intake was associated with thinning of the colonic mucous barrier which was again more pronounced for animal protein as compared with dairy and plant protein [48, 50]. In another rat study, epithelial cell damage and proliferation as well as fecal water cytotoxicity were increased in rats fed soybean protein (20%) versus a casein (20%) diet [53]. In contrast, Vis et al. observed in rats a more protective effect of soy protein (25%) compared with casein (25%) rather than a stimulating effect on colon cancer risk parameters [54].

Recently, rats fed a high-protein diet (53% protein versus 14% in a normal diet) had a marked decrease of the height of the colonocyte brush-border membranes ($p = 0.0001$) which coincided with a higher colonic protease activity ($p = 0.01$) and an increased NH_3 content in the colonic lumen ($p = 0.0008$) [55]. The relationship between the different measured parameters was not further investigated.

Genotoxicity of cecal water from rats was significantly higher after a diet containing barbecued beef as the protein source compared with casein. Protein content in both diets was equivalent to 17% of total diet. The fact that genotoxicity was already detectable in cecal water indicates that passage through the colon is not essential to generate genotoxins. However, as cecal water genotoxicity was not compared with fecal water genotoxicity, it cannot be excluded that further metabolism during passage through the colon could still have increased genotoxicity [56].

4.3 Human studies

4.3.1 Epidemiological studies

Several epidemiological studies [57–59], but not all [60], found an association between meat intake and particularly red meat intake and the risk to develop adenomas or CRC. Only a few epidemiologic studies reported findings for protein intake. A number of prospective studies reported non-significant associations between total consumption of protein and CRC risk [61–64]. In a meta-analysis including three cohort studies and three case–control studies, no significant association was found between animal protein or meat protein and CRC risk [65]. In a recent case–control study in both White and African Americans, the total consumption of protein was associated with a significant risk reduction for distal CRC in Whites and a non-significant association with lower risk in African Americans [66].

Epidemiologic studies are considerably hampered by colinearity between different dietary factors and between dietary and lifestyle factors. Besides protein, red meat intake is associated with saturated animal fat, heme iron or heterocyclic amines (formed during roasting of the meat), all of which have been associated with increased CRC risk [67]. The inability to isolate these factors and analyze them independently makes it extremely difficult to unravel the mechanisms that underlie the association between red meat consumption and CRC. In a consensus report, the World Cancer Research Fund (WCRF)/American Institute for Cancer Research (AICR) concluded that there was convincing evidence supporting a positive association between both red and processed meat intakes and CRC [68]. However, evidence to implicate specific components of meat is inadequate.

The relationship between protein fermentation and inflammatory bowel diseases (IBD) has not directly been investigated. However, several studies examined the association between the pre-illness diet and onset of the disease. Most of these studies were retrospective studies and are therefore prone to recall bias. In a prospective cohort study among $>67\,000$ women, high total protein, in particular animal protein, was associated with a significantly increased risk of Crohn's Disease (CD) and UC [69]. These results were consistent with the observation of Shoda et al. who found a significant correlation between the increasing incidence of CD in Japan and increasing dietary intake of animal protein ($r = 0.908$) [70]. In contrast, a prospective nested case–control study in a sub-cohort from the EPIC study failed to detect an association between UC and diet [71]. In a recent systematic review encompassing 19 studies with 2609 IBD patients and over 4000 controls, high dietary intakes of meat were associated with an increased risk of CD and UC [72].

In addition, in an observational prospective cohort study, high meat (particularly red meat and processed meat) and alcoholic beverage intake predicted an increased likelihood of relapse for UC patients. Red meat contains high amounts of sulfur amino acids, whereas processed foods and alcoholic beverages contain large amounts of sulfite or sulfate [23]. It was suggested that a high sulfur diet results in the generation of H_2S and mucosal damage in the colon. Indeed, untreated UC patients have significantly higher fecal concentrations of H_2S than controls (0.55 mM versus 0.25 mM, $p = 0.027$) [73].

4.3.2 Intervention studies

The Comet assay is often used in intervention studies to explore the genotoxicity of fecal water as a biomarker to study the association between diet and colon cancer [74, 75].

Using this technique, it was shown in healthy subjects that a diet high in fat and meat but low in fiber almost

doubled the genotoxicity of fecal water as compared with a low fat and meat diet [76]. Although animal protein intake was higher in the high fat–high meat diet, the total protein intake was higher in the low fat diet (99.5 g/10 MJ) than in the high fat diet (75.1 g/10 MJ). Shifting from a dairy-rich product to a dairy product-free diet was accompanied with a 30% reduction in protein intake and resulted in a significant increase in cytotoxicity of fecal water, whereas genotoxicity remained unchanged [77]. It was assumed that the dramatic decrease in calcium intake by excluding dairy products was responsible for the increased cytotoxicity.

A strictly controlled dietary trial in 12 healthy male subjects compared fecal water genotoxicity after a 60 g/day red meat diet (containing 65 g protein), a 420 g/day red meat diet (containing 143–150 g protein) and a vegetarian diet (containing 143–150 g protein). No differences in fecal water genotoxicity were found. Although not specified, it is likely that protein fermentation was higher after the 420 g/day red meat and vegetarian diet than after the 60 g/day red meat diet [78].

Benassi-Evans et al. compared fecal water toxicity after a weight-loss diet either high in protein and red meat (35% protein) or high in carbohydrate (17% protein) and also measured fermentation metabolites as biomarkers indicative of bowel health [79]. A significant reduction in total DNA damage was found after 12 wk on intensive weight loss irrespective of the diet, suggesting that active weight loss/caloric restriction has reduced the genotoxic potential of the fecal water. Although fecal phenol or *p*-cresol excretion was not changed, *p*-cresol excretion was weakly correlated with DNA damage, which was considered consistent with their hypothesized role in fecal water genotoxicity. To eliminate the influence of caloric restriction on fecal water genotoxicity, a similar study should be performed in subjects on a non-weight-loss diet.

5 Mechanisms to influence protein fermentation

The most simple strategy to reduce the degree of potentially harmful compounds by protein fermentation is probably a reduction of dietary protein intake [2, 22]. An alternative dietary strategy includes the administration of pre-, pro- or synbiotics.

Dietary interventions with pre-, pro- or synbiotics consistently showed an increase in saccharolytic fermentation while concomitantly decreasing proteolytic fermentation (Table 1).

Administration of resistant starch (RS) to rats significantly decreased urinary *p*-cresol [47] and urinary nitrogen excretion [80]. Provision of fructooligosaccharides (FOS) or xylooligosaccharides to rats decreased urinary nitrogen excretion and increased fecal nitrogen excretion [81].

In humans consuming a high RS diet, fecal ammonia as well as fecal *p*-cresol, phenol and total phenol were significantly decreased [82]. In healthy men, consumption of RS type 3, but not RS type 2, significantly decreased fecal ammonia [80]. Consumption of either lactulose or lactitol for 4 wk resulted in significantly reduced fecal concentrations of phenol, *p*-cresol, indole and skatol [83]. Isomalt consumption did not affect fecal ammonia and *p*-cresol [84].

In our own studies, protein fermentation was reduced after dietary intervention with inulin, oligofructose-enriched inulin (OF-IN), arabinoxylan oligosaccharides and lactulose. To evaluate colonic ammonia metabolism, we administered ^{15}N-labeled ammonia and measured the excretion of the ^{15}N label in urine and feces. Administration of pre- or probiotics stimulates the bacterial assimilation of ammonia, which is reflected in a larger fraction of [^{15}N]NH_3 fixated by the bacteria and excreted in feces and a smaller fraction of the label being excreted in urine [85–88].

In healthy subjects, *Lactobacillus casei* Shirota and *Bifidobacterium breve* (Yakult) significantly decreased urinary *p*-cresol and favorably affected the ammonia metabolism [89]. Also 4 wk *Lactobacillus* GG intake decreased the urinary *p*-cresol excretion [90].

More recently, the impact of dietary interventions has been evaluated using a metabonomic approach which allows evaluating the colonic metabolism from a top-down approach bypassing the need for an a priori hypothesis. Fecal samples were obtained before and after consumption of a synbiotic food (0.5 g FOS, 10^9 CFU *Bifidobacterium longum*, and 10^9 CFU *Lactobacillus acidophilus*, 2 × /day) for 30 days. ^{1}H-NMR analysis and multivariate statistical analysis showed a shift from a more proteolytic to a more saccharolytic which was considered a health benefit for consumers [91]. A similar study characterized the impact of 1 month intake of a synbiotic (0.5 g FOS, 10^9 CFU *B. longum*, and 10^9 CFU *Lactobacillus helveticus*). Metabolites that were positively correlated with the synbiotic administration principally belonged to the ketones and SCFA, whereas the concentration of 1-octanol, thiophene and nonaone significantly decreased after the feeding period [92].

A shift from proteolytic to saccharolytic fermentation was also confirmed after the administration of a synbiotic combination of OF-IN (2 × 10 g/day) and *L. casei* Shirota (2 × 6.5 × 10^9/day) [93]. Decrease of proteolytic fermentation was evidenced by a significant decrease in dimethyl trisulfide and ethyl benzene, a metabolite from phenylalanine degradation [94].

In all of the above-mentioned studies, the observed shift from saccharolytic to proteolytic fermentation was considered as beneficial to health. However, in none of the studies, a parameter of bowel health was measured.

Independent of the studies that showed a decrease in protein fermentation after pre-, pro- or synbiotic administration, a large number of animal studies have shown that treatment with pre-, pro- or synbiotics reduces tumor incidence and precancerous lesions in the colon [95].

Table 1. Effect of intervention with pre-, pro- or synbiotics in humans and animal models on markers of protein fermentation

References	Human/ animal	Subjects (*n*)	Intervention	Product	Dosage	Duration	Parameters	Effects
Younes et al. [103]	Animal	10/treatment	Prebiotic	FOS	7.5% of diet	17 days	Ammonia/N	FOS+XOS: cecal ammonia pool and N ↑
				Xylooligosaccharides (XOS)				FOS+XOS: Urinary N ↓ and fecal N ↑
Heijnen et al. [80]	Animal	18	Prebiotic	RS types 2 and 3	17% of diet	10 days	N	RS2, RS3: urinary N ↓ and RS3: fecal N ↑
Toden et al. [47]	Animal	16	Prebiotic	RS	48% of diet	4 wk	Phenols	Fecal *p*-cresol ↓
Ling et al. [90]	Human	64	Probiotic	*Lactobacillus* GG	2×10^{11} cells	4 wk	Phenols	Urinary *p*-cresol ↓
Birkett et al. [82]	Human	11	Prebiotic	RS (mixture)	39 ± 3 g/day	3 wk	Ammonia	Fecal ammonia ↓, urinary ammonia =
							Phenols	Fecal phenol+*p*-cresol ↓
Heijnen et al. [80]	Human	23	Prebiotic	RS types 2 and 3	32 g/day	1 wk	Ammonia	RS3: fecal ammonia ↓, RS2: no effect
Ballongue et al. [83]	Human	36	Prebiotic	Lactulose Lactitol	2×10 g/day	4 wk	Phenols Indoles	Fecal phenol+p-cresol ↓ Fecal indole+skatol ↓
Gostner et al. [84]	Human	19		Isomalt	30 g/day	4 wk	Ammonia Phenols	Fecal ammonia = Fecal *p*-cresol =
De Preter et al. [104]	Human	30+15	Prebiotic	Lactulose	2×10 g/day or 15 g/day	4 wk	Ammonia	Urinary ammonia ↓+fecal ammonia ↑
De Preter et al. [89]	Human	30+15	Prebiotic	Lactulose	2×10 g/day or 15 g/day	4 wk	Phenols	Urinary *p*-cresol ↓
Geboes et al. [87]	Human	7	Prebiotic	Inulin	3×5 g/day	4 wk	Ammonia Phenols	Urinary+fecal ammonia = Urinary+fecal *p*-cresol =
De Preter et al. [86]	Human	19	Prebiotic	OF-IN	2×10 g/day	4 wk	Ammonia	Urinary ammonia ↓+fecal ammonia =
							Phenols	Urinary *p*-cresol ↓
		19	Probiotic	*L. casei* Shirota	6.5×10^9 cells			Urinary ammonia ↓ (*L. casei* S)+ fecal ammonia =
		10		*B. breve*	10^9 cells			Urinary *p*-cresol ↓
		9	Synbiotic	*L. casei* Shirota+OF-IN	6.5×10^9 cells+ 2×10 g/day			Urinary ammonia ↓+fecal ammonia = Urinary *p*-cresol ↓
Cloetens et al. [88]	Human	10	Prebiotic	Arabinoxylooligosaccharides	2×5 g/day	2 wk	Phenols	Urinary *p*-cresol ↓

Table 2. Effect of consumption of pre-, pro- or synbiotics in human studies on markers of fecal water toxicity

References	Subject (n)	Pre-, pro- or synbiotic	Product	Dosage	Duration	Parameters	Effects
Hosoda et al. [98]	Healthy volunteers ($n = 6$)	Probiotic	*L. acidophilus* LA-2	7.5×10^{10} CFU/day	7 days	FW mutagenicity (Ames test)	↓ after probiotic intake
Osswald et al. [100]	Healthy volunteers ($n = 6$)	Probiotic	*L. casei*	5×10^{9} CFU in 50 g meat/day	9 days	FW genotoxicity (Comet assay)	No effect
Matsumoto et al. [105]	Elderly healthy volunteers ($n = 6$)	Probiotic	*B. lactis* LKM512	5×10^{9} CFU/day	2 wk	FW mutagenicity (umu-test)	↓ after 1 and 2 wk of probiotic intake
Matsumoto et al. [99]	Healthy volunteers ($n = 7$)	Probiotic	*B. lactis* LKM512	5×10^{9} CFU/day	2 wk	FW mutagenicity (umu-test)	↓ after 2 wk of probiotic intake
Oberreuther-Moschner et al. [96]	Healthy volunteers ($n = 9$)	Probiotics	*L. acidophilus*+ *B. longum*	$>3 \times 10^{11}$ probiotic/ day	6 wk	FW genotoxicity (Comet assay)	↓ after probiotic intake
Glei et al. [101]	Healthy volunteers ($n = 38$)	Prebiotic	Inulin	6% in bread	5 wk	FW genotoxicity (Comet assay)	No effect of prebiotic intervention
Rafter et al. [97]	Polyp ($n = 43$) and colon cancer patients ($n = 37$)	Synbiotic	OF-IN	12 g/day	12 wk	FW genotoxicity (Comet assay)	↓ in polyp patients
			L. rhamnosus GG +*B. lactis* Bb12	10^{10} CFU/day		Transepithelial resistance	↑ in polyp patients
						Necrosis	↓ in polyp patients
Worthley et al. [102]	Healthy volunteers ($n = 20$)	Prebiotic	HAMS	25 g/day	4 wk	Proliferation index	No effect
		Probiotic	*B. lactis*	10^{9} CFU/day		Crypt height	No effect
		Synbiotic	RS+*B. lactis*			DNA methylation	↓ methylation of MINT2, and important methylation marker in CRC

Human studies that investigated the effect of pre-, pro- or synbiotic administration on fecal water toxicity are summarized in Table 2. After an intervention in humans with standard yoghurt and yoghurt supplemented with *L. acidophilus* 145 and *B. longum* 913, fecal water toxicity of samples collected from both groups was compared. Fecal water was significantly less genotoxic in the group that received the probiotic yoghurt [96].

In a placebo-controlled randomized trial in polypectomized patients and colon cancer patients, a synbiotic intervention (*Lactobacillus rhamnosus* GG, *B. lactis* Bb12 and OF-IN) for 12 wk resulted in slightly decreased DNA damage as compared with baseline in the polypectomized patients but not in the colon cancer patients. In the polypectomized patients, the intervention also significantly reduced colorectal proliferation and the capacity of fecal water to induce necrosis in colonic cells and to improve epithelial barrier function [97].

In six healthy subjects, administration of milk fermented with *L. acidophilus* LA-2 remarkably decreased (71.9%) fecal mutagenicity compared with baseline [98]. After consumption of *B. lactis*-containing yogurt for 2 wk, mutagenicity level was significantly reduced as compared with the consumption of a placebo ($p = 0.0489$) [99].

Other studies did not show an effect on genotoxicity. Probiotic intervention with *L. casei* for 9 days had no impact on fecal water genotoxicity [100]. Glei et al. evaluated the impact of sourdoughs bread supplemented with prebiotics in 38 healthy male volunteers (20 smokers and 18 non-smokers). Response to the intervention was determined by smoking status and GSTM genotype rather than the prebiotic [101]. A recent cross-over trial of RS and *B. lactis*, alone or combined as synbiotic in 20 human volunteers, failed to show any significant difference in epithelial biomarkers (epithelial proliferation and crypt height) of CRC [102].

6 Discussion and concluding remarks

Environmental factors including diet and lifestyle importantly contribute to the etiology of CRC. As schematically depicted in Fig. 2, several other (dietary and lifestyle) factors than protein fermentation have been associated with increased CRC risk.

In vitro studies using isolated colonocytes or cell lines have confirmed the potential toxicity of individual protein fermentation metabolites such as NH_3 and H_2S. In contrast, the evidence for toxicity of phenolic compounds is rather limited. Whereas urinary excretion of *p*-cresol and phenol has been shown to be a reliable measure of the degree of protein fermentation [22], it remains questionable whether those markers should be considered as a biomarker of bowel health.

Most convincing evidence on potential harmful effects of protein fermentation is derived from animal studies.

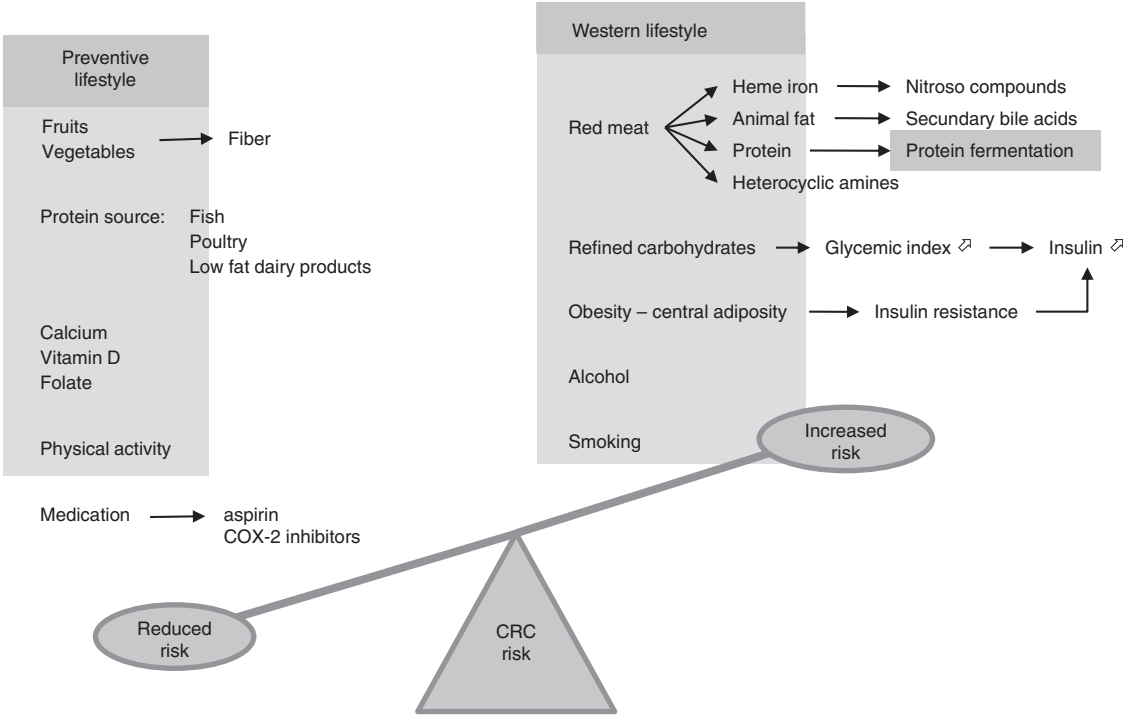

Figure 2. Schematic presentation of dietary and lifestyle factors that contribute to increased and decreased CRC risk (as reviewed by Chan et al. [66]). It is assumed that a combination of factors rather than one specific factor might contribute to increased or decreased CRC risk. The contribution of each individual factor is very difficult to estimate. Protein fermentation is only one of the factors leading to increased CRC risk.

The ability to administer known amounts of different, isolated protein fractions allows to selectively examine the impact of protein fermentation irrespective of other nutrients that are closely associated with the intake of proteins in normal foods such as fat, heme iron or calcium. In rats, high dietary protein intake was associated with increased DNA damage. Since the degree of protein fermentation mainly depends on protein intake [2], it is deduced that high protein fermentation is associated with increased cancer risk. However, only one rat study reported the association between parameters of protein fermentation to parameters of colon cancer risk [47].

The hypothesis that protein fermentation is toxic is further corroborated by intervention studies with pre- and probiotics. Both in rats and humans, those interventions consistently decrease proteolytic fermentation and also decrease the genotoxicity of fecal water, again suggesting a link between protein fermentation and DNA damage. However, this relationship has not been explored. In addition, such an association would not allow presuming a causal relationship.

In contrast, human epidemiologic studies do not support an association between protein intake and CRC. Some studies even suggest a protective effect of non-red meat sources of protein [61]. The absence of such association does at least suggest that other risk factors may be more important. Also, human intervention studies investigating the impact of protein intake on DNA damage failed to proof a harmful effect of protein intake. Unfortunately, parameters of protein fermentation were usually not measured in these studies.

The relation of protein fermentation with other disorders of the large bowel such as IBD and in particular UC mainly focuses on H_2S. It must be emphasized that H_2S in the bowel does not exclusively originate from the fermentation of sulfur-containing amino acids but also from dietary sulfate which is ample present in a Western diet. Nevertheless, from the findings of human studies assessing the impact of diet on UC, it may be speculated that a low protein intake might reduce both the incidence and activity of IBD. Future research will have to resolve whether a low sulfur-containing diet or a low protein diet should be advised.

Considering the whole of available evidence, it is without doubt that protein fermentation yields intrinsically toxic luminal compounds that affect epithelial cell metabolism and barrier function. However, the effects of long-term exposure of the colonic mucosa to those metabolites remain unclear. Available evidence at present seems insufficient to support a role of protein fermentation in the risk of bowel diseases. It is possible that the impact of protein fermentation is overshadowed by other dietary or lifestyle factors. The relative importance of the different factors contributing to increased CRC risk needs to be further addressed.

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