Review

Metabolism of Maillard reaction products by the human gut microbiota – implications for health

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The human colonic microbiota imparts metabolic versatility on the colon, interacts at many levels in healthy intestinal and systemic metabolism, and plays protective roles in chronic disease and acute infection. Colonic bacterial metabolism is largely dependant on dietary residues from the upper gut. Carbohydrates, resistant to digestion, drive colonic bacterial fermentation and the resulting end products are considered beneficial. Many colonic species ferment proteins but the end products are not always beneficial and include toxic compounds, such as amines and phenols. Most components of a typical Western diet are heat processed. The Maillard reaction, involving food protein and sugar, is a complex network of reactions occurring during thermal processing. The resultant modified protein resists digestion in the small intestine but is available for colonic bacterial fermentation. Little is known about the fate of the modified protein but some Maillard reaction products (MRP) are biologically active by, *e.g.* altering bacterial population levels within the colon or, upon absorption, interacting with human disease mechanisms by induction of inflammatory responses. This review presents current understanding of the interactions between MRP and intestinal bacteria. Recent scientific advances offering the possibility of elucidating the consequences of microbe-MRP interactions within the gut are discussed.

Keywords: Colonic microbiota / Gastrointestinal health and disease / Maillard reaction products / Microbial fermentation / Protein glycation

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1 Introduction

Traditionally, the value of food has been measured by its ability to provide energy and nutrients to the host. This process has long been considered to end at the distal ileum and scant attention has been paid to the transformation of dietary constituents that enter the colon. However, from a clinical perspective, the colon was recognised as central to host health with even the earliest of observers, Hippocrates c 400 BC noting that "death sits in the bowel" [1]. Colon cancer is the second leading cause of death from cancer in Western societies and gastrointestinal infections, inflammatory diseases, such as ulcerative colitis (UC) and Crohn's disease, or functional disorders of the gut probably account for the majority of the economic cost of community health care [2–4]. Conversely, there is growing evidence that bacteria within the colon play an important role in maintaining health, providing energy for the host, educating the immune system and maybe even protecting against colon cancer [5]. A wide range of functional foods now exists which target colonic health and this has led to the growth of this industry to a multi-billion Euro market in recent years. In turn, this points to a growing consumer demand and market supply of scientifically sound efficacious products.

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Abbreviations: **AGE**, advanced glycation end-product; **ARP**, Amadori rearrangement product; **CML**, N^{ε}-(carboxymethyl)lysine; **HA,** heterocyclic amine; **IQ**, 2-amino-3-methyl-3*H*-imidazo[4,5-f]quinoline; **MeIQx**, 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline; **MRP**, Maillard reaction product; **PhIP**, 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine; **RAGE**, receptor for advanced glycation end-products; **SCFA**, short chain fatty acids; **SRB**, sulfate reducing bacteria; **UC**, ulcerative colitis

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The human colon is colonized by a complex collection of microorganisms that, in combination with smaller microbial communities on the skin, in the upper regions of the gastrointestinal tract, oral-bucal cavity and the urogenital tract, constitute the human microbiota. Recent estimates put the number of human associated bacterial species at well over 1000, and considering that each of these bacterial species possess their own unique genetic repertoire, with on average 5000 genes, this represents a mega-microbial genome larger than that of the human host [6, 7]. Even allowing for genetic redundancy between different bacterial species within the microbiota, the diversity of metabolic functions encoded by this mega-microbial genome renders the colon a highly metabolic organ. Another important characteristic of human colonic environment is that it operates under predominantly anaerobic conditions. The vast majority of the bacteria within the colon are strict anaerobes (*e.g.* *Bacteroides*, *Clostridium*, *Fusobacterium*, *Ruminococcus*, *Butyrvibrio*, *Bifidobacterium* spp.), which derive energy through fermentation, and more specialized species that carry out other reductive reactions such as methanogenesis, acetogenesis, nitrate reduction and sulfate reduction [8–10]. Higher oxygen tensions may occur at the surface of the colonic epithelial layer, but this has little impact on the strictly anaerobic environment of the colonic lumen as free oxygen is rapidly utilized by facultative anaerobic species (*e.g.* the enterobacteria, lactobacilli, streptococci and enterococci). Anaerobic ecological systems rely on a range of reductive enzyme activities, which operate in the absence of O_2, and bacteria living within anaerobic ecosystems are able to carry out a range of biological transformations not possible under aerobic conditions. The microbiota of the colon derive their energy from dietary compounds which escape digestion in the stomach and small intestine, and endogenous substrates *e.g.* mucins, secreted by the host. Thus, the human colonic microbiota may be viewed as an anaerobic digester, which acts on material recalcitrant to digestion in the upper gut using an array of anaerobic metabolic pathways. This results in metabolites, which may then become available to the host [11]. As discussed below, this transformation of dietary compounds has implications for both human health and disease. However, due to the complexity of the colonic microbiota and difficulties in measuring its metabolic output, the human colonic-systemic metabolic axis has been much understudied.

2 Substrate utilization in the colon

2.1 Carbohydrate

The two main fermentative substrates of dietary origin are non-digestible carbohydrates (resistant starch, non-starch polysaccharides, and fibre of plant origin and non-digestible oligosaccharides) and protein that escapes digestion in the small intestine. Of these, carbohydrate fermentation is more energetically favourable, leading to a gradient of substrate utilization spatially along the colon. The proximal colon is a saccharolytic environment with the majority of carbohydrate entering the colon being fermented in this region. As digesta moves through to the distal colon, carbohydrate availability decreases and protein and amino acids become the main metabolic energy source for bacteria in the distal colon [12]. The principle substrates for bacterial growth are, however, dietary carbohydrates. It has been estimated that about 10 to 60 g/day of dietary carbohydrate reaches the colon. A large proportion of this carbohydrate is made up of resistant starch, *i.e.* starch recalcitrant to the activities of human amylases [13]. The remainder of the carbohydrate entering the colon is made up of non-starch polysaccharides (about 8–18 g/day), unabsorbed sugars, *e.g.* raffinose, stachyose and lactose (about 2–10 g/day) and oligosaccharides such as fructooligosaccharides, xylooligosaccharides, galactooligosaccharides (about 2–8 g/day) [14]. Saccharolytic bacterial species within the colonic microbiota include *Bacteroides* spp., *Bifidobacterium* spp. *Ruminococcus* spp., *Peptostreptococcus* spp., and saccharolytic species belonging to the clostridia like *Roseburia intestinalis* [15, 16]. *Bacteroides* spp. are recognised as versatile members of the dominant microbiota possessing a vast array of polysaccharide hydrolysing enzymes. Recent sequencing of the *B. thetaiotaomicron* genome highlighted more genes involved in carbohydrate acquisition and metabolism than any thus far sequenced bacterium [6, 17]. Other saccharolytic species, such as the bifidobacteria are more efficient at growing on shorter oligosaccharides, whether of dietary origin or by cross feeding off oligosaccharides released by the *Bacteroides* spp. [17, 18]. The rate at which different carbohydrates are broken down by the gut microbiota varies greatly. Simple sugars entering the colon are readily fermented and persist for only a short time in the proximal colon. Some sugars such as raffinose may have a more selective fermentation (being mainly assimilated by bifidobacteria and lactobacilli) while others such as lactose support the growth of a wide range of colonic bacteria. Similarly, non-digestible oligosaccharides reaching the colon display differing fermentabilities and different bacterial species show different glycosidic preferences [17, 19, 20]. Certain oligosaccharides such as fructooligosaccharides and galactooligosaccharides and the polysaccharide inulin, may be fermented preferentially by bifidobacteria, which has given rise to the concept of prebiotics, non-digestible food components that selectively stimulate one or a limited number of beneficial bacteria within the gut [15, 21]. Non-starch polysaccharides include pectin, arabinogalactan, inulin, guar gum and hemicellulose, which are readily fermented by the colonic microbiota, and lignin and cellulose, which are much less fermentable [22, 23]. Endogenous carbohydrates, chiefly from mucin and condroitin sulfate, contribute about 2–3 g/day of fermentable sub-

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strate [8]. Carbohydrates in the colon are fermented to short chain fatty acids (SCFA), principally, acetate, propionate and butyrate [24] and a number of other metabolites such as the electron sink products lactate, pyruvate, ethanol, succinate as well as the gases H_2, CO_2, CH_4 and H_2S [25]. SCFA are rapidly absorbed by the colonic mucosa and contribute towards the energy requirements of the host [26]. Acetate is mainly metabolised in human muscle, kidney, heart and brain, while propionate, a possible glucogenic precursor, is cleared by the liver and suppresses cholesterol synthesis. Butyrate on the other hand is metabolised by the colonic epithelium where it serves as a regulator of cell growth and differentiation [27]. Cross feeding on organic acids occurs within the colon with lactate and acetate being converted to butyrate by specialist bacteria including *Eubacterium* spp., *Faecalibacterium prausnitzii* and *Roseburia intestinalis* and *Butyrivibrio* spp [28, 29].

2.2 Protein

Protein and amino acids are also available for bacterial fermentation in the colon. Approximately 13 g of dietary protein enters the colon daily [30]. Other sources of protein in the colon include bacterial secretions such as enzymes, sloughed off epithelial cells, bacterial lysis products and mucins [31]. Little is known about the species of bacteria responsible for the amino acid fermentation within the gut but certain *Bacteroides* spp., *Eubacterium* spp., *Peptococcus* spp., and clostridia produce a range of proteolytic enzymes [32, 33]. Protein reaching the colon is fermented to SCFA, branched chain fatty acids such as isobutyrate, isovalerate and a range of nitrogenous compounds and ultimately to ammonia [34]. Unlike carbohydrate fermentation, some of these end products may be toxic to the host, *e.g.* ammonia, certain amines and phenolic compounds. High concentrations of ammonia in the colon have been linked to increased DNA synthesis and neoplastic proliferation and in patients with liver disease, can contribute towards the onset of portal systemic enchepalopathy or hepatic coma [35, 36]. Different indoles, amines and phenols that result from amino acid fermentation have been linked to a range of pathologies including schizophrenia, migraine and hypertension [19]. Some of these compounds are directly carcinogenic or are carcinogen precursors, for example, secondary amines and amides act as precursors for the carcinogenic *N*-nitroso compounds [37]. These amines and amides produced mainly by microbial decarboxylation of amino acids may be *N*-nitrosated in the presence of nitrosating agents. Nitrosating agents within the colon include NO released from the colonic mucosa by stimulated macrophages or nitrite derived from dietary nitrate following dissimilatory nitrate reduction by a range of facultative and strictly anaerobic colonic bacteria [38]. Increasing the amount of protein in the diet, even easily digestible protein, can increase production of these potentially toxic bacterial metabolites in the colon [39]. Carbohydrate fermentation is more energetically favourable than amino acid fermentation and occurs preferentially in the proximal colon. As carbohydrate is used up, the relative energetic value of protein increases and the distal colon is a much more proteolytic environment with the many bacteria deriving energy from amino acid fermentation [12]. Excessive protein fermentation, especially in the distal colon, has been linked with disease states such as colon cancer and UC, which generally start in this region of the colon before progressing proximally along the colon. Two major factors govern the amount of protein reaching the colon, the total amount of protein in the diet and the digestibility of this protein in the upper gut. The chemical structure of protein is modified by processing, especially by heating, and this leads to altered chemical structure and hence susceptibility to human proteolytic enzymes.

3 Chemistry of the Maillard reaction

Many components of a typical Western diet, *e.g.* bread, snack items, breakfast cereals, roast meat, cakes, pastries, baked potatoes are heat processed. During heating of such foods, the Maillard reaction occurs and the resulting products contribute importantly to the characteristic flavour and colour [40]. The Maillard reaction is a complex network of reactions and is summarised diagrammatically in Fig. 1. The initial stage of the reaction involves the condensation of a carbonyl group, for example from a reducing sugar such as glucose, with a free amino group such as the epsilon amino group of lysine residues within peptides. This results in the formation of an unstable Schiff base, which spontaneously rearranges to form the more stable Amadori rearrangement product (ARP), also known as fructoselysine (FL) when the initial sugar is glucose. ARP are degraded via various pathways [40, 41], leading to the formation of reductones and furfurals. ARP can be absorbed *in vivo* by diffusion and are metabolized completely by the colonic microbiota [42]. These compounds can react further to give coloured, low molecular mass products and melanoidins, which are brown, macromolecular materials [43 – 45]. ARP (as well as the Schiff base and sugars themselves) can also fragment to form various dicarbonyl compounds, *e.g.* butanedione, glyoxal, methylglyoxal, 3-deoxyglucosone. These are all more reactive than their precursors and can lead to the formation of compounds that contribute to flavour such as Strecker aldehydes (*e.g.* 3-methylbutanal from leucine, methional from methionine), pyrazines, thiophenes and furans [40]. In addition, the dicarbonyl fragments can act as precursors of acrylamide, heterocyclic amines, and low molecular mass coloured compounds and melanoidins.

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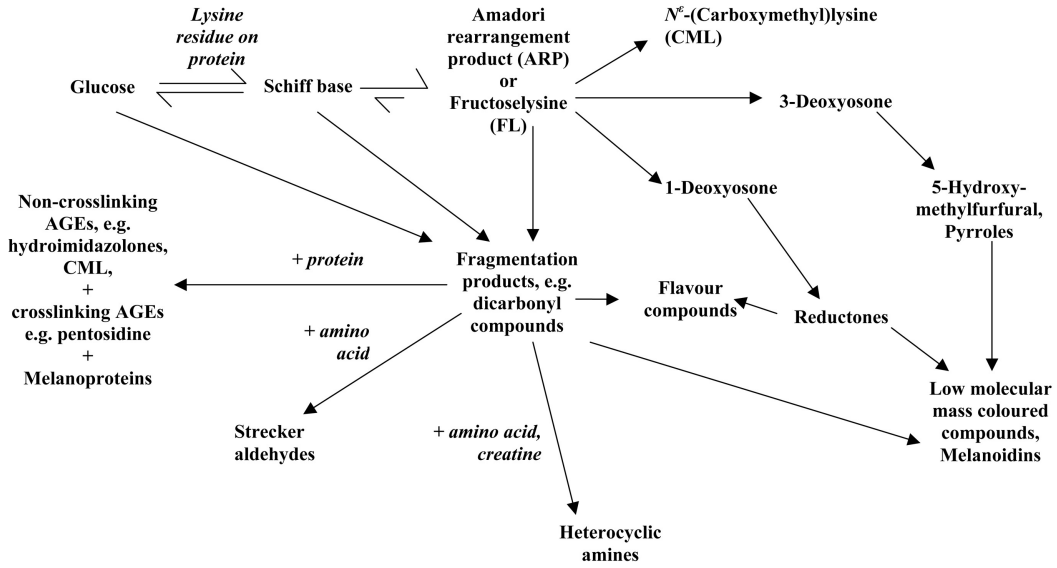

Figure 1. Summary of the Maillard reaction.

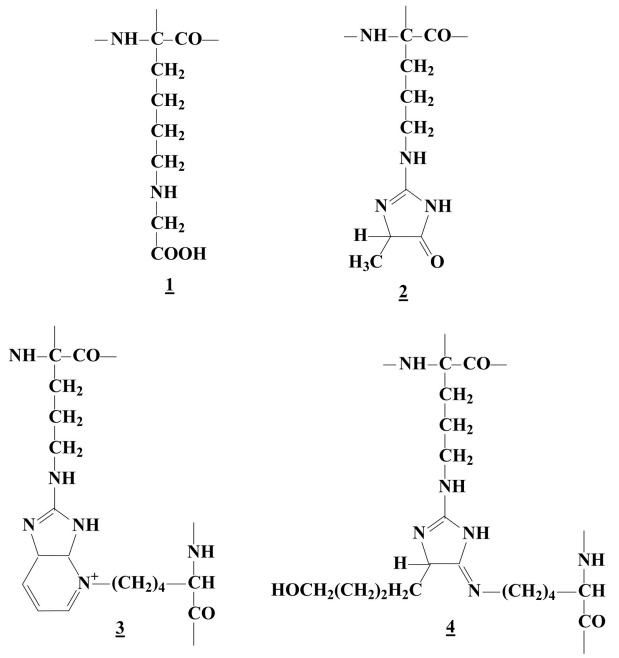

Figure 2. Structures of some advanced glycation end products (AGE). (1) N^{ε}-(Carboxymethyl)lysine (CML); (2) Methylglyoxal-derived hydroimidazolone (MG-H); (3) pentosidine; (4) N^{6}-{2-{[(4*S*)-4-ammonio-5-oxido-5-oxopentyl]amino}-5-[(2*S*,3*R*)-2,3,4-trihydroxybutyl]-3,5-dihydro-4*H*-imidazol-4-ylidene}-L-lysinate (DOGDIC).

Reducing sugars and their carbonyl fragmentation products may also react with the side chains of certain amino acid residues within peptide chains (principally lysine and arginine) to yield advanced glycation end products (AGE). These consist of a diverse range of compounds, several of which have been identified in foods [46]. They include non-cross-linking adducts of lysine, *e.g.* N^{ε}-(carboxymethyl)lysine (CML) [47] and arginine, *e.g.* methylglyoxal-derived hydroimidazolone (MG-H) [48], as well as cross-linking adducts, *e.g.* pentosidine [49] and N^{6}-{2-{[(4*S*)-4- ammonio-5-oxido-5-oxopentyl]amino}-5-[(2*S*,3*R*)-2,3,4-trihydroxybutyl]-3,5-dihydro-4*H*-imidazol-4-ylidene}-L-lysinate (DOGDIC) [50]. The structures of these AGE are shown in Fig. 2. All these protein adducts are colourless but sugars and their degradation products can also form a range of coloured adducts giving cross-linked structures termed melanoproteins [51]. Furthermore, carbonyls formed by lipid peroxidation react with amino acid residues within peptides to give adducts known as advanced lipoxidation end products [52].

4 Implications of MRP for human health

4.1 General remarks

The consequences of dietary MRP for human health may be beneficial or detrimental and have been reviewed very recently [53]. Here we summarise the main implications of MRP for human health and focus on the fate of dietary MRP *in vivo*.

4.2 Loss of nutritional value

When foods undergo the Maillard reaction, losses of essential amino acids, certain vitamins and some metals occur [40]. In addition, the protein may be less readily digested by enzymes. Free and peptide-bound amino acids may be lost

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and, in some foods, losses of the essential amino acid, lysine, may be significant, due to the reactivity of its ε-amino group even when bound in peptide chains. Loss of nutritional value of amino acids occurs once the ARP is formed. These proteins do, however, become available for bacteria within the colon [54] and there can contribute towards increased levels of toxic metabolites following amino acid fermentation.

Following oxidation to dehydroascorbic acid, vitamin C (ascorbic acid) can react with free amino groups in place of the reducing sugar, leading to losses of this vitamin. Certain other vitamins, *e.g.* thiamin, possess a free amino group and can react with reducing sugars in place of the amino acid [55].

Nutritionally important metals such as copper, zinc, and iron may be complexed by Maillard reaction products including ARP and melanoidins [56]. Metals bound in this way are nutritionally unavailable and feeding mixtures of MRP to human subjects leads to increases in levels of metals, *e.g.* zinc in urine.

Another way in which the Maillard reaction may lead to impaired nutritional value of food involves AGE-mediated protein cross-linking. This results in impaired digestibility that may be attributed to reduced access to the proteins by digestive enzymes. For example, trypsin cleaves proteins C-terminal to lysine and arginine residues within peptide chains. If the e-amino group of a lysine residue or the guanidino group of an arginine residue is modified by a carbonyl compound, trypsin loses the ability to cleave and digestion of the protein is reduced. These AGE cross-linked proteins will reach the colon. However, there is little information on the ability of the colonic microbiota to act on such compounds, either fermenting their constituent amino acids or releasing AGE.

A diverse range of compounds with xenobiotic structures are formed during the Maillard reaction in heated foods depending on the amino acid composition of constituent peptides and sugars present in the foods. It is becoming clear that this diverse range of compounds present in our diet have a broad spectrum of effects depending on their individual reactivity, transformation recalcitrance and biological availability. These effects range from pro-carcinogenic or carcinogenic activities to potentially health promoting antioxidants.

4.3 Toxic effects of heterocyclic amines and acrylamide

A number of MRP have been reported to possess mutagenic or carcinogenic properties in animals and/or humans. Model MRP sugar-casein systems have been investigated for their mutagenic activity using the Ames mutagenicity test in *Salmonella typhimurium*. Although very much an experimental model of mutagenic activity, this study showed that the relative mutagenicity of the test MRP was dependant upon the constituent sugar and Maillard reaction mechanism [57]. There is a growing awareness that high dietary consumption of red meat or processed meats increases the risk of developing colon cancer [58]. Cooked meats, especially char grilled red meat or processed meats, contain a range of potentially toxic compounds formed during heat treatment or during subsequent metabolic transformation by human xenobiotic degrading enzymes or by the gut microbiota [59, 60]. Heterocyclic amines (HA) are formed at ppb levels in heated muscle tissue, *e.g.* roasted meat, grilled fish [61] as a result of interactions between amino acids, sugars and creatinine [62]. Although these compounds are unlikely to be the sole cause of colon cancer, they do contribute to the genotoxic or carcinogenic load within the colon. Taken together with other contributory factors such as the toxic metabolites of protein fermentation, haeme-compounds in red meat and increased colonic fat, they may account for the observed increased risk of colorectal cancer associated with red or processed meat [63 – 65]. The most common HA in cooked meats are 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo [4,5-*f*]quinoxaline (MeIQx) and it is estimated that between 43 and 110 ng PhIP and 14 – 47 ng MeIQx are ingested per day for those on a typical Western style diet [60, 66]. PhIP has been shown to cause colonic tumours in a dose-dependant manner in laboratory rats and gives tumours with microsatellite instability similar to that seen in human colonic tumours [67, 68]. MeIQx has also been shown to have strong mutagenic activity in the Ames test and to be a hepatic carcinogen in rodents [69]. To exert their mutagenic or carcinogenic activity these HA must first be transformed into more reactive forms. This may occur after absorption by the hepatic phase I xenobiotic metabolizing enzymes (mainly *N*-oxidation by CYP1A2) [70]. Biotransformation by the phase I enzymes (usually oxidative, reductive or hydrolytic in nature) leads to a range of smaller molecules which can then be excreted through the renal system. This does not necessarily lead to a reduction in toxicity, since structural intermediates can be more reactive than parental compounds and in the case of PhIP and MeIQx, more carcinogenic. The phase II enzymes can also act on dietary xenobiotics such as the HA, leading to the formation of conjugated forms followed by secretion into the intestine via bile. These conjugated forms may in turn be transformed by the colonic microbiota and indeed reabsorbed as the parent compound, which leads to enterohepatic circulation, or excreted in faeces. Bacterial biotransformation of these compounds can also lead to formation of more reactive or toxic compounds. Another HA, 2-amino-3-methyl-3*H*-imidazo[4,5-*f*]quinoline (IQ) produced in lower amounts in

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grilled or fried meats has been shown to be highly mutagenic in the Ames test. Activation of IQ into its directly genotoxic derivative occurs *in situ* in the colon by the bacterial microbiota. Species of *Eubacterium* or *Clostridium* have been shown to catalyse the hydration-dehydration reaction leading to the formation of 2-amino-3,6-dihydro-3-methyl-7*H*-imidazo[4,5-*f*]quinoline-7-one (7-OHIQ) [71, 72]. This directly genotoxic compound is more reactive than IQ and has been shown to be formed *in vivo* and in response to ingestion of foods containing high amounts of IQ but does not occur in the absence of the colonic microbiota [73, 74]. Most HA cause mutations in bacteria and mammalian cells and cancer in animals. The risk to human health of consuming foods containing HA is hard to estimate but, a population-based study performed in Sweden suggests that the normal dietary intake of IQ compounds is unlikely to increase the incidence of cancers of the kidney, bladder, colon or rectum [75].

Acrylamide is carcinogenic in experimental rats and mice [7] and is classified as 'probably carcinogenic' in humans [76, 77]. There are various mechanisms of formation of acrylamide, several involving the Maillard reaction [78]. Asparagine is key to its formation. Highest levels (several hundred μg/kg) of acrylamide have been reported in foods such as French fries, potato crisps, coffee, crisp bread and other cereal products [78]. Nevertheless, the real risk to human health of diet-derived acrylamide remains unclear but a recent review provides a framework for assessing the risk [79]. One important consideration is the fate of acrylamide in the intestine and how its interrelationship with food matrices affects its bioavailability. A recent study has shown that although acrylamide monomers can be absorbed by intestinal cells by passive diffusion, their release from food matrices can be greatly affected by macromolecular food components. For example, acrylamide binds to chicken egg albumin under intestinal and cooking conditions [80]. These authors concluded that such interactions might reduce acrylamide uptake. However, no information exists on the bioavailability of acrylamide carried into the colon within food and whether the colonic microbiota can mediate release of or indeed degradation of this carcinogen.

4.4 Formation of compounds possessing antioxidant activity

A potential health benefit of the Maillard reaction is the observed increase in antioxidant activity in food systems including coffee [81, 82] and malt [83, 84] as well as model systems [85–87]. Both reductones and melanoidins are reported to possess antioxidant activity but interactions between sugars, amino compounds and phenolic acids may result in higher antioxidant activity than that produced by sugars and amino compounds alone [87]. Recently, a novel MRP, 2,4-dihydroxy-2,5-dimethyl-1-(5-acetamino-5-methoxycarbonyl-pentyl)-3-oxo-2H-pyrrole (pronyl-lysine), has been reported in bakery items and is a potent antioxidant [88]. The formation of compounds possessing antioxidant activity in foods may prolong shelf life by inhibiting oxidative reactions resulting in rancidity and off-flavour.

Any health benefits of the compounds will only be realised if they or their metabolites are active at the biological target site. Using *in vitro* experiments, it has been demonstrated that fructosyl arginine [89] and aminoreductones [90] formed by the Maillard reaction protect low-density lipoprotein (LDL) against oxidation. A few studies have examined the effects of MRP on antioxidant enzymes. Pronylated BSA and pronyl-glycine are both inducers of chemopreventative enzyme activity in Caco-2 intestinal cells [88]. In contrast, glycated casein has been shown to have no significant effect on antioxidant enzyme activities in Caco-2 cells [91]. However, activities of glutathione peroxidase, glutathione reductase and superoxide dismutase were all significantly decreased in Int-407 cells following a challenge with glucated or fructated, but not ribated, casein. No feeding studies have been reported to date concerning the ability of MRP to protect against LDL oxidation or to upregulate antioxidant enzymes.

4.5 Fate of dietary MRP *in vivo*

Virtually all reports of the fate of dietary MRP *in vivo* concern AGE. When the diet consists largely of processed foods such as heated milk, baked cereal items such as bread, and coffee, the intake of ARP has been estimated to be 0.5–1.2 g/day while the intake of AGE (mainly CML and pyrraline) might be 25–75 mg/day [46]. Restriction of diet-derived AGE leads to a significant lowering of circulating AGE in both animals and humans [92]. The metabolic transit of dietary ARP and AGE has been reviewed [93]. Components may be (i) released from protein by digestive enzymes or gut bacteria and absorbed; (ii) metabolised by bacteria in the gut; and (iii) excreted in the faeces.

Absorbed components may be excreted in urine or accumulate in tissues. Rat and human studies have shown that 3–10% of administered protein-bound ARP is excreted in the urine with a further 1–3% being excreted in faeces [93] and <80% being degraded by the gut microbiota. The amount that accumulates in the body is unknown [93]. Much less is known about the fate of food AGE [93] although CML has been identified in the urine of human infants. Feeding ^{14}C- or ^{3}H-labelled casein-sugar model systems to rats [94, 95] resulted in a 16–22 % recovery of radioactivity in urine after administering labelled glycated protein, compared to 10% for labelled unglycated protein. The faecal recovery was 3–4% for the unheated material and up to 40–50% for

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the glycated protein, depending on the extent of heat treatment [93]. A smaller amount of radioactivity was detected in the liver, muscles and kidneys of animals fed glycated casein compared to the unglycated protein. More recent studies [92] using a nonspecific ELISA and human subjects have shown that ~10% of dietary AGE are absorbed and result in a proportional rise in serum AGE level such that the contribution of dietary AGE to total plasma AGE peaks at 50% in serum following consumption of a high AGE diet. Only about 30% of diet-derived serum AGE were excreted in the urine of healthy subjects within 48 h. The precise fate of the remainder is unknown.

AGE are able to bind to the receptor for AGE (RAGE), expressed on the surface of various cells including endothelial [96], kidney [97] and Caco-2 (human colon adenocarcinoma) [98]. Ligation of AGE by RAGE induces the generation of reactive oxygen species (ROS) by an NAD(P)H oxidase. The free radicals in turn activate a Ras-MAP-kinase pathway, which eventually leads to the activation and translocation of NF-κB [99]. Induction of NF-κB leads to the transcriptional activation of many genes including a variety of relevance for inflammation such as tumour necrosis factors (*e.g.* TNF-a) and interleukins 1, 6 and 8. The resulting inflammatory situation ultimately leads to disease. Dietary AGE or increased serum concentrations of AGE, have been implicated in a number of pathologies including renal insufficiency, dyslipidemia, diabetes and ageing. There is also strong evidence linking high serum concentrations of AGE and pathologies associated with diabetes mellitus, including vascular and renal dysfunction [92, 100 – 102].

UC is a chronic inflammatory disease of the intestinal tract and, unlike Crohn's disease, is usually limited to the colon. Pathology usually starts in the distal colon before moving proximally towards the small intestine. There is no cure and maintenance therapies are not always successful, with surgical intervention the only remaining course of action in many cases. The aetiology of UC is unknown but is considered to be multi-factorial with predisposing genetic, and environmental contributory factors [103, 104]. Diet, the gut microbiota and interactions between diet and the gut microbiota provide the environmental stimuli within the colon and have been implicated in both aetiology (*e.g.* toxic, pro-inflammatory metabolites and toxigenic bacteria such as the sulfate reducing bacteria, SRB) and protection from disease (*e.g.* probiotics microorganisms and prebiotics) [105 – 107]. Colonic degradation of MRP provides a number of potential mechanisms, which may contribute to inflammatory processes within the colonic mucosa, including increasing the toxic metabolites of amino acid fermentation, release of AGE within proteinaceous food matrices and stimulation of more harmful species of colonic bacteria.

5 Measuring the metabolism of MRP by gut microbiota

A range of *in vitro* and *in vivo* models of the human gastrointestinal microbiota may be applied to study the interactions between MRP and the gut microbiota [108]. *In vitro* systems range from simple anaerobic pH controlled batch cultures to three-stage compound continuous culture models using human faecal biota as inoculum [108, 109]. These systems have the advantage that they may be used to determine the MRP-degrading capabilities of the gut microbiota with easy access to the metabolic end products of such transformations [110]. To study the potential of endogenously formed MRP present in heated foodstuffs to undergo microbial conversion within the colon, simulated digestion using mammalian amylolytic, lipolytic and proteolytic enzymes followed by dialysis through semi-permeable membranes may be used. These digested MRP may then be used as test substrates in the *in vitro* culture systems [111]. There are a number of reasons one would carry out such experiments *in vitro* rather than *in vivo*. First, the *in vitro* culture systems allow the investigator to determine the metabolic end products of microbial transformation of MRP, while such end products may be absorbed *in vivo* and remain undetected in faeces. Similarly, these *in vitro* culture systems employ a human inoculum, which is important considering the significant compositional and metabolic differences between the gut microbiota of humans compared to laboratory animals [108]. Finally, *in vitro* culture systems offer a cost effective experimental tool for looking at the microbial conversion of model MRP that are often only available in small quantities, and not in the quantities necessary to carry out meaningful animal or human feeding studies. Gnotobiotic technologies, including ex-germ free animals colonized with human intestinal microbiota, overcome some of the limitations of the *in vitro* systems, in that they also include a mammalian input towards metabolic conversions and absorption of end products. The health implications of microbial biotransformations may be more readily measured because of the availability of mucosal samples from different regions of the gut. Such studies have been central to identifying the microbiota as key players in the conversion of cooked food mutagens into their more carcinogenic derivatives [112, 113]. They also offer the possibility of post-mortem examination of tissues for specific pathologies, *e.g.* pre-cancerous lesions on the intestinal mucosa, and experimental designs, which include interventions with toxic or carcinogenic substances. Such studies have also been employed to measure the potential of different potential functional foods to protect against these cooked food mutagens [114]. However, they are expensive and studies on the fate of MRP in existing food products can proceed from initial *in vitro* screenings directly to human feeding studies on target populations [115 – 117]. A number of recent advances in the fields of microbial ecol-

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Table 1. Numbers (log 10 bacterial count) of selected groups of bacteria following fermentation of BSA and enumeration using species-specific 16S rRNA targeted probes with fluorescent *in-situ* hybridisation (FISH)[a)]

	Healthy subject			Ulcerative colitis patient		
Bacterial group	Native BSA	Heated BSA	Glycated BSA	Native BSA	Heated BSA	Glycated BSA
Lactobacilli	8.07 ± 0.02	7.83 ± 0.08	7.90 ± 0.03	7.51 ± 0.02	7.44 ± 0.04	7.33 ± 0.02
Bifidobacteria	8.75 ± 0.04	8.64 ± 0.02	8.51 ± 0.09	8.11 ± 0.02	8.07 ± 0.01	7.94 ± 0.06
Bacteroides	8.97 ± 0.01	8.89 ± 0.02	8.94 ± 0.02	8.94 ± 0.03	9.01 ± 0.02	9.07 ± 0.04
Eubacterium rectale	8.84 ± 0.01	8.53 ± 0.08	8.76 ± 0.05	8.66 ± 0.05	8.67 ± 0.01	8.40 ± 0.02
Clostridium perfringens/histolyticum	6.83 ± 0.06	7.13 ± 0.02	7.14 ± 0.02	8.99 ± 0.20	9.00 ± 0.03	9.09 ± 0.60
Sulphate-reducing bacteria	7.76 ± 0.07	7.75 ± 0.06	7.84 ± 0.01	8.01 ± 0.02	8.05 ± 0.02	8.15 ± 0.04

a) Data are from one healthy subject and one ulcerative colitis (UC) patient and were obtained from samples taken from vessel 3, simulating the distal colon, of an *in vitro* gut model.

ogy, nutritional molecular biology and analytical technologies are now revolutionising the way we can study interactions between the diet, human metabolism (including metabolic activities of our resident microbiota) and disease susceptibility. Molecular microbiological techniques taking advantage of the phylogenetic information encoded by the 16S rRNA gene present in all bacteria allow the microbiologist to capture species diversity and visualise population fluxes within the complex gut microbiota in a manner never before possible with traditional culture based bacteriological techniques [7, 10]. We can now identify and enumerate specific bacterial species directly within environmental samples in a phylogenetic relevant manner without the need for cultivation, and in a much more rapid manner. Advances in molecular nutrition and disease are identifying biomarkers of disease risk, which means that changes in these biomarkers measurable in body fluids, urine, blood and faeces, can be linked to reduced risk of developing diseases like colon cancer and heart disease [118–120]. Such chronic diseases often develop over decades and are not amenable to intervention studies using absence of disease as the biological end point.

Melanoidins and AGE-modified proteins formed via the Maillard reaction are only partially digestible by endogenous enzymes. Studies on both rats and human diabetic subjects have indicated that as little as 10% of immunoreactive dietary CML is transported to the circulation [92, 121]. The first work investigating the ability of gut microorganisms to ferment dietary MRP used individual species of bacteria [122]. Our early work demonstrated that numbers of anaerobic species increased following fermentation of MRP prepared from glucose and lysine using total faecal bacteria in batch culture [111]. A three-stage laboratory gut model of the human colon that has been validated against sudden death victims [123] is a means of studying the effect of dietary components on the growth of colonic bacteria (obtained from a faecal sample from a human subject) in mixed culture. We are currently investigating the effects of dietary MRP on the profile of colonic bacteria using both the *in vitro* gut model and an *in vivo* dietary intervention trial with human subjects. The *in vitro* investigation involves cultivation of faecal bacteria on native BSA or heated BSA or glycated BSA. Glycated BSA is prepared by incubating a solution of BSA (1 mM) and glucose (0.4 M) in 0.2 M pH 7.4 phosphate buffer at 50°C for 24 h. Control BSA is prepared in the same way but omitting the glucose. Enumeration of bacteria is carried out using species-specific 16S rRNA targeted probes with fluorescent *in-situ* hybridisation (FISH). A comparison of the profile of bacteria obtained from faecal samples from both ulcerative colitis patients and healthy subjects is being performed. Initial data for one healthy patient and one ulcerative colitis subject and relating to the area of the gut model that mimics the distal region of the colon are shown in Table 1. *In vitro*, numbers of the more detrimental species of bacteria, including clostridia and SRB, are higher, at the expense of bacteria seen as more beneficial, *i. e*. the bifidobacteria and lactobacilli, following fermentation of native protein by a faecal sample from the ulcerative colitis patient. In general, compared to native BSA, the heated protein resulted in higher numbers of the more detrimental bacteria and lower numbers of the bifidobacteria and lactobacilli. This effect was accentuated when glycated protein was the substrate. This preliminary observation would be consistent with increased persistence of the glycated-BSA into the third vessel of the gut model and a resultant increased amino acid fermentation. However, these microbiological observations must be confirmed in replicates using faecal inocula from other healthy and UC subjects and *in vivo*. Similarly, to determine the implications of an altered microbial profile in this region of the colon, metabolic profiling will be carried out on *in vitro* cell free supernatants and *in vivo*, using faecal water. The effect of both model MRP and MRP in real foods on expression of key xenobiotic degrading enzymes will be determined using Caco-2 cells.

Increased protein fermentation within the distal colon would lead to the production of a range of toxic metabolites, phenols, branch-chain fatty acids, amines and amides the

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subsequently, N-nitroso compounds, and ammonia which is directly toxic and proliferative. Van Nuenen *et al*. [124] using an *in vitro* model of the colonic microbiota (the SHIME reactor) observed increased branch chain fatty acids, phenols and ammonia in the region of the model mimicking the distal colon, when inoculated with faeces from UC subjects compared to models inoculated with faeces from healthy subjects. Increased SRB would also indicate that sulfate reduction was increased with subsequent production of the directly cytotoxic H_2S. The SRB have already been linked with UC, and *Desulfovibrio* spp. isolated from the intestine of UC patients produce ten times more H_2S than similar strain isolated from healthy individuals [105]. Similarly, AGE are an integral part of glycated proteins and may up-regulate inflammatory pathways via RAGE [100, 125]. Such toxic and pro-inflammatory events are thought to contribute either to the onset or maintenance of UC, an inflammatory bowel disease, which invariably starts in the distal colon before progressing proximally causing mucosal necrosis.

6 Conclusion

The metabolic output of the human colonic microbiota is increasingly understood to play a central role in maintaining human health and conversely, when things go wrong, to initiate chronic diseases like colon cancer and UC. The metabolic versatility of this diverse microbiota acts in concert with human metabolic systems to transform a range of dietary compounds, including phytochemicals, drugs, and xenobiotic compounds [126]. Some of these metabolic processes lead to the production of compounds, which improve human health directly by acting as antioxidants for example, or by detoxification of harmful compounds. However, they have also been shown to produce more toxic derivatives of xenobiotic compounds, which enter the colon through our diets. The Maillard reaction is responsible for production of a large variety of structurally diverse xenobiotic metabolites upon heat treatment of foods. Some of these MRP have been shown to possess antioxidant activities while others are thought to play a role in colon toxicology, through increased colonic protein fermentation, and some such as the AGE may also act as systemic toxicants and inducers of inflammation. To date, little is known about the relationship between our intestinal microbiota and MRP or their biological activities. Both *in vitro* and *in vivo* systems are now being employed to address this situation and together with recent advances in molecular microbial ecology, disease biomarker identification and application of high resolution analytical techniques (*e.g*. LC-MS, ^{1}H-NMR), promise to elucidate some of the secrets behind microbe-MRP interactions [11].

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