Review

Evidence against dietary advanced glycation endproducts being a risk to human health

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In vivo, advanced glycation endproducts (AGEs) are linked to various diseases, particularly those associated with diabetes. AGEs are also formed when many foods are thermally processed. The extent to which dietary AGEs are absorbed by the gastrointestinal (GI) tract and their possible role in the onset and promotion of disease are currently of considerable interest. This paper reviews information that supports the argument that dietary AGEs are not a risk to human health.

Keywords: Advanced glycation endproducts / Amadori rearrangement products / N^{ε}-(carboxymethyl)lysine / Maillard reaction / Pyrraline

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This article focuses on contra arguments about "dietary AGEs are a risk to human health".
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1 Introduction

Humans have cooked food since the discovery of fire. Cooking, by whatever means, *e.g.* boiling, grilling, roasting, frying, results in the destruction of bacteria with the potential to cause food poisoning as well as the elimination of heat-sensitive toxins, including, *e.g.* lectins and protease inhibitors [1]. Furthermore, cooking may lead to an increase in the bioavailability of nutrients, especially protein derived form some plant sources. Cooking also results in the formation of the typical colour and flavour of many items of a typical Western diet, including coffee, bakery items and roasted meat. Thus, cooking food contributes importantly to enjoyment of life.

Over the last 10–15 years, attention has been directed towards the possible undesirable effects of components that result from cooking. One class of such compounds are advanced glycation endproducts (AGEs) that form when reducing sugars react with compounds possessing a free amino group, notably lysine residues within protein. Sugars may oxidise to dicarbonyls, *e.g.* glyoxal and methylglyoxal which are particularly reactive towards arginine residues in protein to form hydroimidazolones. When a reducing sugar reacts with the epsilon amino group of lysine, a reaction intermediate, known as an Amadori rearrangement product (ARP) is formed. ARPs are pre-AGEs and when the initial reducing sugar is glucose, the ARP is known as fructoselysine (FL). ARPs are unstable reaction intermediates that degrade to AGEs, including those reported to be the most abundant representatives in food, *i.e.* N^{ε}-(carboxymethyl)-lysine (CML) and pyrraline [2]. AGEs comprise a diverse group of compounds and the structures of ~20 have been elucidated to date. So far, most research on AGEs have been directed to their effects *in vivo*, where they are associated with the complications of diabetes, especially renal, retinal and cardiovascular disease [3].

The structures of AGEs that have been most studied in the context of food and nutrition are shown in Fig. 1. It has been estimated that consumption of a typical Western diet results in a daily intake of 1.2 g of ARPs (calculated as FL) and 25–75 mg of AGEs (mainly CML and pyrraline) [2].

This paper first reviews information obtained from animal and human studies concerning the bioavailability and metabolic fate of dietary ARPs and AGEs. This is followed by a discussion of the bioactivity of AGEs. As far as possible, examples are drawn from studies with human subjects and from investigations in which AGEs have been quanti-

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Abbreviations: AGEs, advanced glycation endproducts; **ARPs,** Amadori rearrangement products; **CEL,** N^{ε}**-(carboxyethyl)lysine; CML,** N^{ε}**-(carboxymethyl)lysine; FL,** fructoselysine; **GI,** gastrointestinal; **LL,** lactulosyllysine; **SRB,** sulphate reducing bacteria

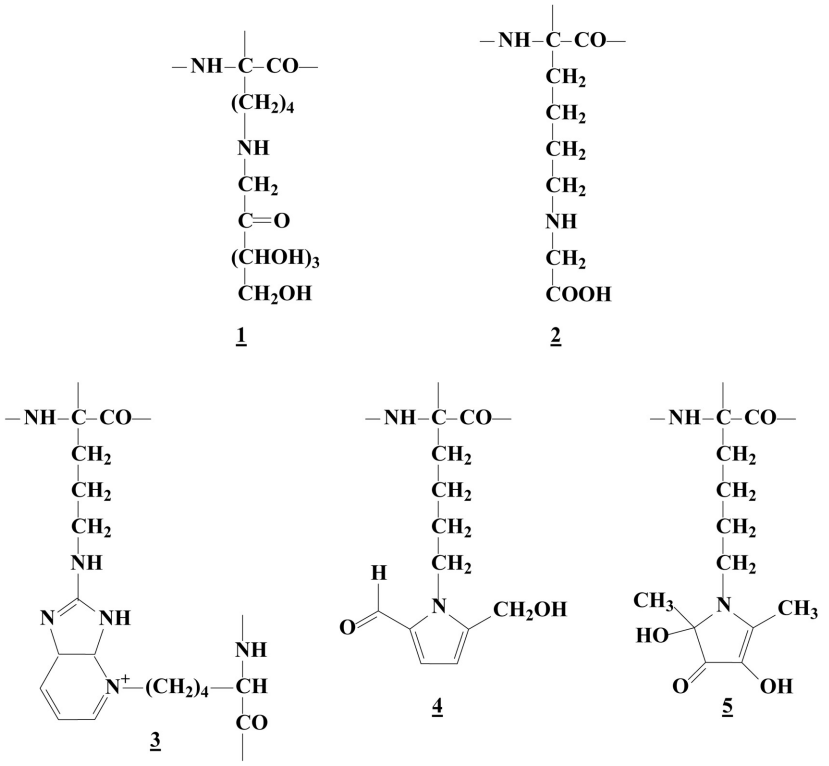

Figure 1. Structures of FL and selected dietary AGEs. 1, Fructoselysine (FL); 2, N^{ε}-(carboxymethyl)lysine (CML); 3, Pentosidine; 4, Pyrraline; 5, Pronyllysine.

fied using highly specific instrumental methods. The goal is to provide evidence that dietary AGEs are not a risk to human health.

2 Bioavailability and metabolic fate of dietary ARPs and AGEs

The human body may be divided into two compartments, *i.e*. the gastrointestinal (GI) tract and the remainder of the body. Dietary components may exert bioactivity in both of these compartments but it is a requirement that in order to exert bioactivity in the body apart from the GI tract, components must first be absorbed. Postabsorption, the metabolic fate of the compounds is of interest, including the kinetics of elimination from the body. Thus, studies reported in the literature have focused on bioavailability of dietary ARPs and AGEs using both animal studies and experiments with human volunteers. The findings for ARPs, CML, pyrraline and pentosidine are summarized in Table 1.

2.1 ARPs

The first studies on protein-bound ARPs involved either feeding rats radiolabelled protein-bound ARPs [4], or human dietary intervention studies in which volunteers were asked to consume glycated casein [4, 5]. These studies revealed that ARPs must be released by proteolysis prior to absorption in the GI tract by passive diffusion rather than active transport. Around 3–10% of dietary ARPs are excreted by the kidneys with only 1–3% being recovered in faeces [5]. Studies with rats have shown a proportion of dietary ARPs to be deposited in various organs, such as the liver, kidneys and pancreas, but recoveries are very low and the kidney is the main site of deposition [4]. In addition, the vast majority (up to 80%) of dietary ARPs is degraded by bacteria that reside normally in the large intestine. For example, *E. coli* can catalyse the ATP-dependent phosphorylation of FL. The resultant FL-6-phosphate subsequently undergoes reversible conversion to glucose-6-phosphate and lysine [6].

Since the major route for excretion of ARPs from the body appears to be the kidney, it is conceivable that dietary ARPs (and AGEs) might accumulate in plasma of renal patients. A recent acute human dietary intervention study [7] on the metabolic transit of the ARP, lactulosyllysine (LL), focused on analysis of plasma and urine. Subjects were in three groups (healthy volunteers, subjects with renal failure but not on dialysis and patients following a dialysis regime). After following a low AGE diet for 5 days, subjects consumed either milk containing 453 mg of LL or a control milk. Analysis of 24 h urine samples showed that the excretion rate of LL was only ~2% in healthy people. In patients with renal failure but not on dialysis, ~1% of dietary LL was recovered in urine while plasma levels did not rise. Even in dialysis patients, plasma LL levels did not

Table 1. Summary of the bioavailability and metabolic fate of ARPs, CML, pyrraline and pentosidine

	Bioavailability	Metabolic fate	Recovery in urine[a)]	Recovery in faeces[a)]
ARPs	Limited	Up to 80% metabolized by colonic microflora. Proportion of the absorbed amount deposited in kidneys	<10%	1–2%
CML	Uncertain, possibly up to 75%	Proportion of the absorbed amount deposited in kidneys and liver. ~50% not accounted for	26–29%	15–22%
Pyrraline	High	Not metabolized postabsorption	~80%	nd[b)]
Pentosidine	High if peptide-bound, low if in the free form	Metabolized postabsorption to uncharacterized compounds	~2%, if mainly in the bound form; ~75% if mainly in the free form	nd

a) Recovery of consumed amount.
b) Not determined.

change significantly and nor did the LL urinary excretion rate. The recovery of LL in faeces was only 1–2% and although the authors comment that the deglycating enzyme, fructoseamine-3-kinase, may play a role in the metabolism of ARPs, they consider that large amounts of ARPs are likely to undergo metabolism by the colonic microflora.

The use of radiolabelled ARPs and AGEs greatly aids understanding of the metabolic fate of these compounds because metabolites as well as the parent compound may be tracked. Intravenous injection of ARPs permits the study of the likely fate of ARPs absorbed from the GI tract. Free ARPs and small peptides containing ARP residues are the forms of ARPs most likely to be absorbed by the gut wall. A recent study involved the intravenous injection of 4-[^{18}F]fluorobenzoylated N^{ε}-FL, a model of small peptides containing FL residues, into the tail vein of rats [8]. Animals were sacrificed at 5 and 60 min after injection. Sixty minutes after injection, ~55% of the radioactivity had been excreted in urine and ~35% had been deposited in the kidneys. The conversion of 4-[^{18}F]fluorobenzoylated N^{ε}-FL to N^{α}-[^{18}F]fluorobenzoylated N^{ε}-(3-phosphofructosyl)lysine by fructoseamine-3-kinases was demonstrated but this metabolite accounted for only 1.7% of radioactivity in urine 60 min postinjection, with 91.9% of the radioactivity being accounted for by 4-[^{18}F]fluorobenzoylated N^{ε}-FL [8].

2.2 AGEs

Most work on the bioavailability and metabolic fate of dietary AGEs has involved CML and pyrraline, both of which are abundant in food. One study [9] involving feeding rats for 10 days with casein glycated to different extents (1.8 g of CML/kg diet or 6.2 g of CML/kg diet) or native casein has shown large amounts of dietary CML to be recovered in urine (accounting for 26–29% of the amount consumed) and faeces (accounting for 15–22% of the amount consumed). Around 1.4% of dietary CML was recovered in kidney tissue and low levels were also deposited in liver. Around half of the CML consumed was not accounted for and based on recovery data for ARPs from the organs of rats following feeding studies, it seems unlikely that deposition would account for a large proportion of the total amount consumed. It is possible that CML is degraded by the colonic microflora but to date this has not been investigated. CML might be metabolized postabsorption but a study involving feeding radiolabelled CML would be required to establish this and no such studies have been reported.

The metabolic fate of intravenously injected radiolabelled CML has been monitored [10]. [^{18}F]-Fluorobenzoylated lysine, CML and N^{ε}-(carboxyethyl)lysine (CEL) were injected into the tail vein of rats and their distribution and elimination were monitored. Animals were sacrificed 5–30 min after injection. The kinetics of distribution to different organs and elimination by the kidneys was the same for lysine, CML and CEL. The components were deposited mainly in the kidney, bladder and liver. One-third of initial radioactivity was measured in urine 5 min after injection and >87% of radioactivity was excreted by the kidneys within 2 h of injection. This rapid and high excretion rate implies that any interaction with body proteins will be low.

The metabolic fate of dietary pyrraline has been investigated, using an intervention study with 18 healthy volunteers [11]. Subjects followed their usual diet (containing 3.1–6 mg pyrraline) on days 1 and 5 and a low AGE diet on days 2–4 of the study. Analysis of 24 h urine samples established that almost all (~80%) dietary pyrraline is absorbed and then rapidly excreted *via* the kidneys within 48 h. The same authors later extended their studies to include the metabolic fate of pentosidine [12]. The length of the study was increased to 9 days and on days 2–8 the 18 healthy volunteers followed a low AGE diet. On day 5, the volunteers were divided into four groups and members of each group

consumed a test meal of either pretzel sticks, coffee, custard (containing defined amounts of AGEs) or continued with the control diet. Urinary excretion of pyrraline and FL fell by ~90% when the control diet was followed but the decrease in urinary excretion of pentosidine was only ~40%. About 50% of pyrraline but only 2% of pentosidine from pretzel sticks was recovered in urine while 60% of pentosidine from coffee was recovered. Pentosidine was almost exclusively peptide bound in pretzel sticks whereas 75% of pentosidine was in the free form in coffee. This resulted in pentosidine from coffee being excreted within 24 h while pentosidine from pretzel sticks was excreted over 72 h. When radiolabelled pentosidine was administered intravenously to rats, 80% of the radioactivity was recovered in urine within 72 h, but only 20% of the radioactivity was attributed to pentosidine, the remainder being due to uncharacterized metabolites [13]. This study indicates that the vast majority of dietary pentosidine will be rapidly excreted.

3 Bioactivity of dietary AGEs

Even when ARPs and AGEs are absorbed by the body, they may not cause harm. The evidence presented above indicates that the majority of the proportion that is absorbed is rapidly excreted. There is a lack of studies concerning the bioactivity of dietary AGEs. One group of papers resulting from acute and chronic feeding studies with well-defined diets [14–16] and animals and human subjects report positive correlations between dietary AGEs and plasma AGEs and insulin resistance, positive correlations between postprandial serum AGE levels and markers of inflammation induced by RAGE, and prolonged urinary excretion of dietary AGEs in renal patients compared to healthy controls. However, there is uncertainty about the specificity of the antibody that was used for all of these studies and there are doubts about the linearity of response of the assay over a wide AGE concentration range.

Other studies have monitored the effect of a Western diet *versus* a prudent diet [17]. The Western diet contained higher levels of red and processed meats, sweets, deserts, potato chips (French fries) and refined grains and is likely to contain higher levels of AGEs than the prudent diet, which was characterized by higher levels of fruits, vegetables, legumes, fish, poultry and whole grains. Positive correlations were reported between the Western diet and plasma proinflammatory markers C-reactive protein (CRP), IL-6, E-selectin, sICAM-1. However, care must be taken in drawing conclusions from such studies concerning the effects of dietary AGEs because any interdiet effects might be due to non-AGE differences, for example, differences in levels of dietary antioxidants.

In the absence of studies concerning the bioactivity of dietary AGEs, investigations of the bioactivity of endogenously formed AGEs might shed light on the possible effects of diet-derived compounds. One hypothesis concerning the possible adverse effects of dietary AGEs is that they increase levels of plasma proinflammatory markers and oxidative stress. A cross-sectional study of 312 stable haemodialysis patients [18] investigated the relationship between serum CRP and serum CML as a predictor of all mortality and cardiovascular mortality. The investigation concluded that the best survival rates were for patients with low CRP and high CML levels, with high CML levels possibly reflecting a better nutritional status. High serum CML was not linked to increased mortality in haemodialysis patients. Another study [19] could find no relationship between serum CML and cardiovascular events and renal outcomes in patients with type 2 diabetes.

Studies to date suggest that dietary AGEs that are absorbed by the gut wall do not adversely affect human health but it is worth investigating the possibility that the ARPs and AGEs that are not absorbed have an adverse effect on GI health. With this in mind, we conducted a pilot randomized dietary intervention crossover study in which five healthy subjects adhered to either a low AGE diet or a high AGE diet for 2 wk. Following a 2 wk washout, they then adhered to the other diet for 2 wk. The intervention was, *per* day, crumb from eight slices of white bread supplemented by mildly heated foods (low AGE diet) or six slices of toast prepared from the same bread supplemented by severely heated foods (high AGE diet). Faecal samples were taken before and at the end of each leg of the diet and colonic bacteria were enumerated using 16S rRNA probes and fluorescent *in situ* hybridization. We were particularly interested in investigating the effects of the two diets on levels of bifidobacteria and sulphate reducing bacteria (SRB). Bifidobacteria are able to ferment prebiotic oligosaccharides, leading to beneficial health outcomes for the human host. In contrast, the products of SRB metabolism include hydrogen sulphide, which is cytotoxic and SRB have been associated with relapse in patients with the inflammatory bowel disease, ulcerative colitis. However, the unpublished data indicate that diet had no significant effect on the numbers or classes of bacteria in this preliminary study.

Some studies suggest that certain dietary AGEs might have beneficial bioactivity. Pronyllysine is a modification of lysine that has been identified in foods including bread crust and malt [20, 21]. When rats were fed for 15 days, a diet that was supplemented with bread crust, malt, or BSA with a pronyllysine content of 0.29% w/w (pronyl-BSA), the activities of the Phase II enzymes, glutathione-*S*-transferase (GST) and UDP-glucuronyl-transferase (UDP-GT), but not the Phase I enzyme, NADPH-Cytochrome-*C*-reductase, were increased in liver and kidney [21]. The 18% increase in GST activity in kidney of animals fed bread crust and 27% increase in UDP-GT activity liver of animals fed pronyl-BSA were statistically significant. Furthermore, significant increases in plasma total antioxidant capacity

and significant decreases in plasma thiobarbituric acid reactive substances (TBARs) were observed after oral administration of bread crust, malt or pronyl-BSA [21].

4 Conclusions

Research on dietary AGEs is patchy, there are many knowledge gaps and questions to be answered. There is a need for studies on animals and humans using carefully defined model diets and meals as well as real meals. Most likely, only a small fraction of dietary AGEs has been characterized and almost all studies involve CML. Although the metabolic transit of only three AGEs, *i.e*. CML, pyrraline and pentosidine, has been investigated, the large difference in recovery in urine of the compounds (from 2 to 80%, depending on the AGE and whether it is consumed in the free or protein-bound form) makes it clear that further work is required to establish the fate of other dietary AGEs, especially those that are reported to be abundant in food such as methylglyoxal-derived hydroimidazolone [2]. In addition, good animal models are required to obtain meaningful data from chronic dietary intervention studies. Certain groups of people might be at risk from dietary AGEs. These include infants, where the GI tract is relatively permeable, to allow absorption of antibodies, and people suffering from inflammatory bowel disease. Since absorbed dietary AGEs are excreted *via* the kidney, it is reasonable to expect those with renal failure to also be at risk. Nevertheless, the few studies undertaken so far suggest that this is not the case.

In conclusion, the data to date suggest that dietary AGEs are not harmful to human health. This is partly because the efficiency of absorption is generally much less than 100%. Up to 80% of dietary ARPs are not absorbed but are degraded by the gut microflora. The majority of the AGE compliment of foods that is absorbed is rapidly excreted by the kidneys. There is no solid evidence obtained using human subjects for a positive relationship between intake of dietary AGEs and upregulation of proinflammatory markers or changes to the profile of the colonic microflora. However, there is some evidence for beneficial effects of dietary AGEs, *i.e*. raised levels of Phase II enzymes and antioxidant activity and decreased plasma TBARs.

Jenny Ames holds a BSc in food science from Reading University and a PhD in flavour chemistry from London University. Following a career spanning 18 years at Reading University, she moved to Ireland in 2005 where she is Professor of human nutrition and health at Queen's University Belfast. Her research currently focuses on the impact of thermally treated foods on human health and disease. Jenny is President-Elect of the International Maillard Reaction Society.

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