# 22 Modified Amino Acid-Based Molecules: Accumulation and Health Implications

**S. Bengmark***
*Division of Surgery and Interventional Science, University College London, London, UK*

## 22.1 Abstract

Industrial processing of food has not only improved the management and safety of foods, but also its taste. Unfortunately however, most of these processes – including plant breeding, gene manipulation, fractionation, separation, condensation, drying, freezing, heating, irradiation, roasting, microwaving, toasting, smoking, emulsification, and homogenization – appear to be negative, as they reduce the nutritional quality of the food and also contribute significantly to increased vulnerability to development of diseases, especially those referred to as endemic and chronic.

This chapter deals especially with the negative consequences of heating and mainly with the impact of heat-produced glycated and lipoxidated molecules, often referred to as Maillard products. These products are more specifically referred to as advanced glycation end-products (AGE) and advanced lipoxidation end-products (ALE). The negative effects on health of other heat-produced compounds, such as heterocyclic aromatic amines, are outside the scope of this review.

Modern molecular biology has made it possible to explore the impact of these and other process-induced molecules on the body and its functions. The detection in 1992 of a specific receptor in the body for such products provided the opportunity for a better understanding of their effects in health and disease. This receptor for advanced glycation end products (RAGE) is recognized as a key member of the immunoglobulin superfamily of cell surface molecules. It functions as a master switch, induces sustained activation of NF-κB, suppresses a series of endogenous autoregulatory functions, and converts long-lasting pro-inflammatory signals into sustained cellular dysfunction and disease. Its activation is associated with much increased levels of dysfunctioning proteins in body fluids and tissues, and is strongly associated with a series of diseases from allergy and Alzheimer's disease to rheumatoid arthritis and urogenital disorders. It is important to observe that heat treatment and other forms of processing of foods will dramatically increase the content of these dysfunctional molecules, and thereby, with time, significantly contribute to the epidemic of chronic diseases seen around the world. An increased

* E-mail address: stig@bengmark.se

  *Amino Acids in Human Nutrition and Health* (ed. J.P.F. D'Mello)

consumption of raw foods, fruits, and vegetables; foods rich in polyphenols and other antioxidants; as well as live bacteria, probiotics, and plant fibre seems appropriate in order to counteract these undesirable developments.

## 22.2 Introduction

Modern food is often extensively processed prior to distribution and sale. Drying, freezing, heating, irradiation, roasting, microwaving, toasting, emulsifying, homogenizing, and the addition of numerous compounds are all aimed at enhancing the appeal of the food, its palatability, and its shelf-life. The effects of each of these manipulations on human health are not fully explored, and even when documented, often not considered to the extent they should be, either by industry or the consumer.

Heating food to higher temperatures is generally regarded to improve both the taste and smell of the foods we eat. High temperature makes food proteins change structure: coagulate, aggregate, and produce crusts. Modern food chemists, chefs, and cooks use this information every day to produce delicious new foods.

The French biochemist Louis-Camille Maillard described the chemical process which occurs non-enzymatically in foods (Maillard, 1912), and which is much accelerated by heating. This process is now referred to as the Maillard reaction and its products collectively named Maillard products. Reducing sugars (fructose, glucose, glyceraldehyde, lactose, arabinose, and maltose) will during the process bind to amino acids, nucleic acids, including DNA, RNA, peptides, and proteins, to produce transitional compounds, most often referred to as Amadori products. In time these undergo complex changes: cyclization, dehydration, oxidation, condensation, cross-linking, and polymerization, to form irreversible chemical products. In particular, reactive carbonyls such as glyoxal and methylglyoxal have been found to rapidly modify reactive side chains of proteins. Important amino acids such as lysine (essential amino acid) and histidine (essential for children) are often involved. During the process significant amounts of pigments (melanoids) but also thousands of often good-tasting and good-smelling so-called volatile compounds will be released. These pigments often make the food or parts of the food look brown or black, which is why the process is sometimes referred to as 'browning'. Common browning products are bread crusts and roasted surfaces of fried meat and fish, all sorts of broths, irrespective of vegetable or animal origin, and all smoked food, as well as Asian sauces, balsamic products, Chinese soy, and cola products, all rich in brown/black Maillard products. But not all Maillard products are dark in colour; there are also white Maillard products, especially dairy products such as cheese and powdered milk. Maillard himself suggested that the Maillard process might be negative to health, as these products will accumulate in the body, as we now know, for many years and sometimes for the rest of life. The process might also reduce the availability in the body of important and essential amino acids.

## 22.3 Effects of Heating on Food Quality

Heat-induced alterations of foods are considered to commence at around 28°C – the highest processing temperature allowed for olive oil to be called virgin. Most enzymes in foods become deactivated after approximately 42°C. Some antioxidants are resistant to heat but a majority will disappear in the interval 30°–100°C, and almost all during microwaving. The heat-enhanced production of Maillard products – glycated and lipoxidated molecules – is said to start and accelerate from around 80°C. Similarly the heat-induced production of carcinogens such as heterocyclic amines is said to start from the interval 100°–130°C, after which the production accelerates dramatically.

Maillard products based on association of carbonyl groups in sugars and proteins are collectively referred to as AGE. Similar products, formed between reactive fatty acids and proteins, are referred to as ALE. Numerous such synthetic products are now identified, but two or three previously unknown compounds are each year added to the list. The most commonly studied AGE

are pentosidine, $N^{\varepsilon}$-carboxymethyl-lysine (CML), and $N^{\varepsilon}$-(carboxyethyl)lysine (CEL).

It is important to note that the production of both AGE and ALE are not dependent on enzymes. The intensity in their production increases, not only with increase in temperature, but also with length of storage at elevated temperatures, and even at room temperature. The content of the AGE furosine increases dramatically with heat treatment such as pasteurization and especially with the production and storage of powdered milk (Baptista and Carvalho, 2004). Other industrial practices commonly used in food processing such as irradiation, ionization, microwaving, and smoking also contribute significantly to increased production of AGE/ALE. Vegetable-based foods are no exception – industrial treatment of plant products, such as roasting, drying, and 'curing' will lead to amounts of AGE/ALE that are as great as those found in animal products. One such example is roasted peanuts.

Fresh tobacco leaves, fresh coffee beans, and fresh peanuts are rich in powerful antioxidants, most of which will disappear during the industrial process ('curing', roasting) and be replaced by often large amounts of AGE/ALE. As the temperature increases above 130°C, carcinogens, especially heterocyclic amines, will also be produced, and their production increases dramatically as the temperature increases. AGE/ALE will not exclusively reach the body through the food we eat, or the smoke we inhale – they are also produced spontaneously in the body, especially in the presence of increased levels of sugars and fatty acids in body fluids and tissues, and in those suffering chronic diseases such as diabetes and chronic renal diseases.

## 22.4 AGE/ALE Accumulation in the Body

The accumulation of late/matured Maillard products – AGE/ALE – in the body is in principle irreversible; what is accumulated in the tissues persists for a very long time and most often forever. Hitherto, the finding of larger amounts of AGE/ALE in the tissues of elderly individuals has simply been regarded as a normal effect of ageing. However, it might not be so. Instead it might mainly be a result of the lifestyle chosen, smoking, and eating habits, and thus in theory preventable. Large to extreme increases in AGE/ALE are regularly observed in body fluids and tissues of patients with chronic diseases, particularly those with diabetes and chronic renal diseases, and in patients suffering complications of these diseases. It is commonly observed in diabetic patients, who suffer from reduced wound healing (Peppa *et al.*, 2003), retinopathy, nephropathy (Zheng *et al.*, 2002), and angiopathy (Vlassara *et al.*, 2002; Lin *et al.*, 2003). Accumulation of AGE/ALE in tissues is seen as intracellular or extracellular deposits referred to as tau proteins, amyloid β proteins (Smith *et al.*, 1994), and in neurofibrillary tangles (Smith *et al.*, 1994; Vitek *et al.*, 1994). Such depositions in various body tissues were long regarded as degenerative but biologically inert structures. However, increasing evidence supports the conclusion that these structures are foci with very strong pro-inflammatory potential, which maintain the systemic chronic inflammation at a high level in the tissues, and thereby accelerate further production of AGE/ALE and exacerbation of disease.

## 22.5 Modern Molecular Biology: Essential for Understanding the Effects of AGE/ALE

Almost 100 years ago, Maillard suggested that accumulation in the body of AGE/ALE would significantly contribute to the progression of diseases, especially of chronic urinogenital diseases, and in particular uraemia. He created what he called an 'index of urinogenital imperfection', which he used to document the association between the degree of accumulation in the body of Maillard products and the severity of disease, especially chronic renal disease.

The time was, however, not opportune for such radical thinking, and the concept was largely ignored by scientists and clinicians of the time, remaining so for several decades to come. It was the introduction of modern molecular biology and particularly the identification of specific receptors in the body for these

substances that would dramatically change the attitude and interest in these substances by both biologists and physicians. The turning point seems to be the identification in 1992 by the American Ann Marie Schmidt of a specific receptor for AGE/ALE named RAGE (Schmidt *et al.*, 1992, 1993, 1994a,b). Since then increasing numbers of publications have appeared in the literature. From the year 2000, several international scientific organizations have become involved in the concept, arranging special symposia on RAGE and AGE/ALE, and publishing special issues relating to these topics. New societies have also been founded with the aim of specifically investigating the effects of food-derived AGE/ALE on health and well-being. The New York Academy of Science seems to have taken the lead and many scientific contributions on AGE/ALE are published each year in its *Annals*. Searches on PubMed relating to AGE and ALE reveal more than 5000 publications, of which more than 25% appeared in 2009. In addition almost 20,000 titles on PubMed are about glycated haemoglobin, $HbA_{1c}$.

Several methods are available for the measurement of content of AGE/ALE in body fluids and tissues: immunohistochemistry with polyclonal or monoclonal antibodies, high performance liquid chromatography (HPLC), and mass spectrography. A majority of these substances are auto-fluorescing even if not visible to the human eye, and so can be used for diagnostic purposes (Meerwaldt *et al.*, 2005a,b). The fluorescence has its maximum at wavelengths between 350 and 440 nm (Meerwaldt, 2005b). Often-studied substances such as CML and CEL do not, unfortunately, show any fluorescing ability, nor do they have any colour. Despite this, measuring fluorescence is an excellent tool for clinical use, especially for screening of individuals with suspected high levels of AGE/ALE in the body, but also for screening of foods suspected to be rich in such dysfunctional proteins.

## 22.6 RAGE: a Master Switch and Key to Inflammation

RAGE is a prominent member of what has been called the immunoglobulin superfamily of cell-surface molecules. It is described as a 'master switch' with the ability to coordinate the inflammatory reaction in the body. RAGE induces a long-lasting activation of the pro-inflammatory transcription factor NF-κB and suppresses a series of endogenous auto-regulatory functions (Schmidt *et al.*, 2001; Bierhaus *et al.*, 2001, 2005a; Vlassara, 2005). Increased deposition of AGE/ALE in the tissues is suggested to be a key element in the development of the so-called metabolic syndrome (Koyama *et al.*, 2005; Soldatos *et al.*, 2005). Accumulation of AGE/ALE and subsequent activation of RAGE is reported to induce a significant down-regulation of leptin in adipose cells (Unno *et al.*, 2004). RAGE activation induces effects on a great variety of tissues, but they are particularly pronounced in endothelial cells, where increased expression occurs of a long row of molecules such as VCAM-1, ICAM-1, E-selektin, eNOS, and TGF-β. TNF-α, Il-6, PAI-1, and VEGF are seen (Bohlender *et al.*, 2005). Strong RAGE-induced effects are often observed on immune cells, macrophages (Sunahori *et al.*, 2006), and dendritic cells (de Leeuw *et al.*, 2005; Ge *et al.*, 2005), but also on smooth muscle, particularly in the walls of blood vessels, under the mucosa and in the skin (Aronson, 2003). These changes are associated with subsequent reduction in regenerative capacity and function of the cells, increased blood pressure, and development of chronic diseases and/or exacerbation of complications to chronic diseases (Monnier *et al.*, 2005). However, the conditions vary from tissue to tissue, the most sensitive and vulnerable being those with low regenerative capacity and long-lived cells such as myelin- and collagen-rich structures, where the substances are likely to remain. Among these are brain, peripheral nerves, skeleton, muscles, tendons, joints, skin, and eye, especially the lens.

Research in recent years has also demonstrated the existence of an endogenous soluble form of RAGE known as sRAGE, which has an important effect as a decoy for RAGE and has been shown to prevent accumulation of RAGE in body tissues (Bierhaus *et al.*, 2005b). This suggests that chronic diseases are not only associated with increased levels of RAGE in the body, but also, and probably as important, with low levels of sRAGE.

## 22.7 Factors Underlying Enhanced Systemic Inflammation

The largest part of the immune system is, in contrast to what was earlier believed, to be found in the gastrointestinal (GI) system (Brandtzaeg *et al.*, 1989) as 70–80% of the Ig-producing cells are located within the GI tract. This explains why the food we eat has such a profound influence on our well-being and health. Although AGE/ALE seem to play a major role, it is also clear that numerous other food-related factors will influence the degree of systemic inflammation in the body, the sensitivity to develop disease and our daily well-being. Increasing evidence suggests that all these factors are additive and collectively contribute to development of a sustained, long-lasting, often discrete and unrecognized, exaggerated stage of inflammation in the body, commonly seen before and when a chronic diseases is manifest. Such factors are:

- *Low vitamin D status*. There is a strong correlation between the level of vitamin D in the body, degree of inflammation and incidence of chronic diseases. Individuals living at higher latitudes such as Canada, Russia, and Scandinavia, but also countries in the Southern hemisphere, such as Argentina, New Zealand, and Uruguay are reported to have generally lower levels of vitamin D in serum, especially during the winter season. This phenomenon is associated with higher incidence of coronary–vascular diseases, acute coronary events (Zittermann *et al.*, 2005; McCarty, 2005) and other chronic diseases such as cancer (Mohr *et al.*, 2006, 2007, 2008).
- *Low levels in the body of antioxidants such as folic acid and glutathione, and increased levels of homocysteine*. Increase in serum levels of homocysteine is regularly associated with increased levels of systemic inflammation and chronic diseases (Mattson, 2003).
- *Impaired hormonal homeostasis*. Ageing as well as chronic diseases are commonly accompanied by hormonal disturbances of various kinds, sometimes to the extent that ageing has been referred to as a state of 'hormonal chaos' (Hertoghe, 2005). Hormonal disturbances are often accompanied by increased oxidative stress/increased release of free radicals, intracellular accumulation of 'waste products', inhibition of apoptosis, disturbed repair mechanisms, reduced gene polymorphism, premature shortening of telomeres, and reduced immune defence. Reduced resistance to disease is often observed in premature ageing as well as in several chronic diseases (Hertoghe, 2005). In particular, 17β-estradiol, plentiful in dairy products, is known to induce a strong activation of RAGE mRNA in endothelial cells. This effect is abolished if an anti-oestrogen such as 4-OH tamoxiphen is supplied (Yamagishi *et al.*, 1998, Suzuma *et al.*, 1999). An impaired hormonal homeostasis is suggested to explain why chronic diseases are often aggravated during pregnancy, frequently seen as vascular and eye complications to diabetes (Suzuma *et al.*, 1999). Physical as well as mental stress also contributes to activation of RAGE, and increased release of noradrenaline is reported to reduce immune defence and increases sensibility to acquire infections by up to 4 logs (Cooper, 1946). Increased release of noradrenaline in the intestine will dramatically reduce the beneficial intestinal flora, and increase the virulence of potentially pathogenic micro-organisms (Kinney *et al.*, 2000; Alverdy *et al.*, 2003), changes, which most likely also contribute to increased RAGE activation. Permanently increased levels of noradrenaline are reported in chronic diseases such as Alzheimer's disease and also found to correlate well with severity of the disease (Peskind *et al.*, 1998). Parathyroid hormones constitute another example of hormones deeply involved in the inflammatory process. Significant elevations in IL-6 are observed in hyperparathyroidism as in other chronic conditions with increased systemic inflammation such as obesity (Flyvbjerg *et al.*, 2004).
- *Angiotensin/renin*. Oxidative stress and increased systemic inflammation is also strongly associated with increased release

of angiotensin, increased levels of free fatty acids in serum, and with reduction in beta-cell function in diabetes (Flyvbjerg *et al.*, 2004; Tikellis *et al.*, 2006; Allen *et al.*, 2005). The observation that blockage of the angiotensin receptor will reduce production and accumulation of AGE both *in vitro* and *in vivo* is of great interest (Allen *et al.*, 2005).

- *Larger intake of glutenoids*. Glutenoids are increasingly regarded as pro-inflammatory in the body (Tlaskalová-Hogenová *et al.*, 2005), and suggested to occur even in the absence of intestinal changes (Brady and Hoggan, 2002; Sbarbati *et al.*, 2003).
- *Low intake of plant antioxidants*.
- *High intake of carbohydrates*.
- *High intake of saturated and trans fatty acids*. A strong association is repeatedly documented between the average content of fat in food and morbidity and/or mortality in chronic diseases in a country, as demonstrated for breast cancer (Carroll, 1975), as well as other cancers and also chronic diseases such as coronary heart disease (Artaud-Wild *et al.*, 1993; Moss and Freed, 1999) and diabetes (Dahl-Jorgensen *et al.*, 1991). More than three quarters of the saturated fat consumed is of bovine origin, and thus it is not surprising that the incidence of various chronic diseases also correlates well with the amount of dairy products consumed (Ganmaa *et al.*, 2002).

## 22.8 Dietary Choice

The incidence of most chronic diseases has increased dramatically during the last 150 years, much of it in parallel with the significantly altered intake of foods which has occurred since the year 1850: a doubling of intake of saturated fat, 50% reduction in intake of omega-3 fatty acids, and a more than doubling in intake of omega-6 fatty acids (Leaf and Weber, 1988). The intake of refined sugar has during the same time period increased from approximately 0.5 kg to about 50–60 kg per person per year. Furthermore, the transition in use to carbohydrates with stronger pro-inflammatory effects such as high-fructose corn syrup (HFCS), seems to make the situation even worse. In the United States, the intake of HFCS in carbonated drinks and fast foods now exceeds that of sucrose (Gaby, 2005).

A recent study in mice is of particular interest. Over 4 months, RAGE knock-out (KO) mice received either a standard diet (7% fat) or a Western 'fast-food'-like diet (21% fat) and were compared to wild-type mice, receiving the same diet. The Western-food-like diet was associated with significant cardiac hypertrophy, inflammation, mitochondrial-dependent superoxide production, and accumulation of AGE in both strains, but significantly less in the RAGE-KO mice. Both strains demonstrated reduced levels of inflammation and oxidative stress, in association with reductions in AGE as well as RAGE on supply of an AGE inhibitor (alagebrium chloride, 1 mg $Kg^{-1}$ $day^{-1}$ (Tikellis *et al.*, 2008).

Much can be learnt from studies of Japan, which has during the last 50–60 years made similar, although not as extensive, changes in food habits as the West. The incidence of several chronic diseases has increased dramatically during this time. As an example, the incidence of prostate cancer has increased 25-fold during the last 50 years, much in parallel to an increase in the consumption of industrially produced agricultural foods: 7 times more eggs, 9 times as much meat, and 20 times as much dairy product (Ganmaa *et al.*, 2002, 2003).

## 22.9 Dairy in Focus

Commonly, 10–20% and sometimes up to 70% of the amino acid lysine is reported to be modified during the common industrial treatment of milk (including sterilization, pasteurization, and irradiation). Fructoselysine is the dominating modified molecule, but CML and pyrraline are also produced during the processing of milk. Sugar content, the level and time of elevated temperature, and storage time are the main factors behind the increased production of AGE/ALE in milk products.

Not only the industrial treatment of dairy products but also the feeds given to the cows

have changed dramatically during the 20th century – from mainly forage-based to starch-rich and fast-absorbed carbohydrates such as corn, maize grains, barley, molasses, and dextrose. Intensive feeding of cows with carbohydrates induces insulin resistance in animals as well as in humans. Should the animals be allowed to live long enough they would also show the same symptoms of Western diseases including manifest diabetes. It has been demonstrated that milk- and lactose-fed calves show signs of insulin resistance at a young age (Hostettler-Allen *et al.*, 1994).

High levels of pro-inflammatory cytokines and various stress hormones are regularly observed in intensively fed animals. No information is available in the literature, however, to support the notion that elevated inflammatory molecules are transferred to humans by meat and dairy products from such animals. Today's dairy products come, much in contrast to the old days, up to about 80%, from pregnant cows, and consequently are rich in growth factors and various hormones, especially sex hormones (Malekinejad *et al.*, 2006), some of which (like 17β-oestradiol) are potentially pro-inflammatory and carcinogenic. It is suggested that dairy-derived hormones and growth factors are important pathogenetic factors behind the development of hormone-dependent cancers, especially of the colon, prostate, and breast (Outwater *et al.*, 1997). These hormones follow the fat fraction and are thus more concentrated in condensed products such as butter, cheese, and powdered milk.

It has been demonstrated that vegans, in great contrast to meat-eaters and lacto-vegetarians, have lower levels of AGE/ALE. Lacto-vegetarians seem to have even higher levels of AGE/ALE than meat-eaters (Sebekova *et al.*, 2001), which might be explained by a higher intake of dairy products, especially cheese, to compensate for not eating lean meat, but which might also be due to a higher intake of fructose. Significant health advantages are reported for vegans: lower levels of pro-inflammatory molecules, cytokines and acute phase proteins; lower systolic and diastolic blood pressure; lower total cholesterol; lower LDL-cholesterol; lower fasting blood sugar and triglycerides; and lower incidence of chronic diseases, especially diabetes and complications to diabetes (Barnard *et al.*, 2009). It would be no surprise if the lowest levels of AGE/ALE are to be found in the group referred to as raw-eaters, especially if they avoid dairy-based foods, but unfortunately, this group has attracted few studies and none with regard to the content of AGE/ALE.

## 22.10 AGE/ALE and Disease

Increased accumulations of AGE/ALE in tissues have been reported in numerous chronic diseases, as detailed below. In addition, changes in the skin and oral cavity may serve as markers of health risks associated with AGE/ALE.

### 22.10.1 Allergy and autoimmune diseases

Thermal processing, curing, and roasting of foods are known to often increase allergenicity of pre-existing allergens and also to introduce new antigens. Sometimes, however, reduced allergenicity has been reported (Davis *et al.*, 2001; Sancho *et al.*, 2005). Common foods such as milk, peanuts, and soy are reported to induce significant increases in AGE levels and to severely affect the IgE-binding capacity (Chung *et al.*, 2001; Franck *et al.*, 2002; Rautava and Isolauri, 2004). Significantly elevated urinary levels of the AGE pentosine are observed in allergic children in association with signs of exacerbation of atopic dermatitis (Tsukahara *et al.*, 2003).

### 22.10.2 Alzheimer's disease and other neurodegenerative disease

Alzheimer's disease (AD) is one of the most common chronic diseases, affecting approximately 5% of all individuals over 65 years of age and more than 35% of those over 80. Strong similarites exist between AD and type 2 diabetes (T2DM), to the extent that Alzheimer's has been called 'the diabetes of the brain', or type 3 diabetes. The incidence of AD is reported to to be increased two- to fivefold in TD2DM (Nicolls, 2004). An approximate threefold

increase in content of AGE is observed in AD brains compared to age-matched controls (Moreira *et al.*, 2005). A common feature of both diseases is accumulation of amyloid deposits, a process which progresses during the course of disease, and much relates to the stage of disease. Signs of amyloidosis, pertubation of neuronal properties and functions, amplification of glial inflammatory response, increased oxidative stress, increased vascular dysfunction, increased Aβ in the blood–brain barrier, and induction of autoantibodies are regularly seen. Increased levels of AGE and signs of oxidative damage are almost regularly observed in the eyes, known to be early targets of AD (Moreira *et al.*, 2005). Central to, if not the cause of AD, is the progressive oligomerization and deposition in the cells of amyloid β-peptides (Aβ), tau, prions, and transthyretin, all glycated molecules with strong neurotoxic effects. Amyloid β-peptides accumulate extracellularly to form amyloid plaques, while tau protein deposits occur as neurofibrillary tangles within the cells. Increased levels of AGE/ALE are most often demonstrated with immunohistochemical methods in senile plaques, tau proteins, amyloid β proteins, and in neurofibrillary tangles (see below (Vitek *et al.*, 1994; Moreira *et al.*, 2005)). Accumulation of AGE/ALE in brain tissues has also been observed in Parkinson's disease (PD) (Castellani *et al.*, 1996; Dalfo *et al.*, 2005), and cytoplasmic proteinaceous inclusions composed of the protein α-synuclein (α-syn) and named Lewy bodies are regularly observed in PD. AGE/ALE are also implicated in the pathogenesis of other neurodegenerative diseases: amyotrophic lateral sclerosis (ALS) (Chou *et al.*, 1998; Kikuchi *et al.*, 2002; Kaufmann *et al.*, 2004), Huntington's disease (Ma and Nicholson, 2004), stroke (Zimmerman *et al.*, 1995), familial amyloidotic polyneuropathy (Gomes *et al.*, 2005), and Creutsfeldt-Jakob disease (Sasaki *et al.*, 2002). Early accumulation of AGE is also reported in Down's syndrome, and early antiglycation treatment is suggested to reduce cognitive impairments (Odetti *et al.*, 1998; Thiel and Fowkes, 2005). It has also been suggested that bovine spongiform encephalopathy (BSE) a disease with its significant similarities to AD, might be associated with increased glycation and lipoxidation (Frey, 2002). Involvement of glycation products and activation of prion proteins are also suggested by other authors (Boratynski and Gorski, 2002; Choi *et al.*, 2004). AGE, amyloid fibrils, and prions all seem to have the same target, RAGE, and all activate the NF-κB pathway. Interaction between RAGE and Aβ is most likely to be the most important implication in the development of AD, enhancing inflammation in blood vessel endothelium, inducing increased response of NF-κB, mediating transport of Aβ across the blood–brain barrier, suppressing cerebral blood flow, and inducing cell death (apoptosis). RAGE is known to mediate Aβ-induced migration of monocytes across the thin brain endothelium and into the brain tissues.

Increased cholesterol is suggested to contribute to the production of AD by increasing generation of beta-amyloid (Aβ), and animal studies suggest that cholesterol co-localizes with fibrillar Aβ in the amyloid plaques (Burns *et al.*, 2003).

### 22.10.3 Atherosclerosis and other cardiovascular disorders

Oxidative stress, lipid peroxidation, and protein glycation are repeatedly associated with extensive arteriosclerosis. Significant increases in both chemical AGE (carboxymethyllysine) and fluorescent AGE (spectrofluorometry) were observed in 42 patients with atherosclerosis when compared to 21 healthy controls ($p<0.001$) (Kalousova *et al.*, 2005). Increased levels of malondialdehyde, lipid peroxides, and pentosidine were seen in a study of 225 haemodialysis patients and these also correlated significantly with the degree of coronary artery calcifications (Taki *et al.*, 2006). Significant lipid oxidation, deposition of AGE/ALE in the arterial walls, and development of atherosclerosis, are reported in rabbits fed a diet containing 1% cholesterol. Deposition is further enhanced when 10% fructose is added to the diet (Tokita *et al.*, 2005). Structural modifications of high density lipoproteins (HDL), lipoxidation, glycation, homocysteinylation, or enzymatic degradation will make HDL lose its anti-inflammatory and cyto-protective ability (Ferretti *et al.*, 2006). This emphasizes its

importance in the pathogenesis of arteriosclerosis, as in neurodegenerative diseases, diabetes, and other autoimmune diseases (de Leeuw *et al.*, 2005). Supplementing AGE-modified serum albumin to experimental animals will significantly increase secretion of pro-inflammatory cytokines, maturation of dendritic cells, and augment the capacity to stimulate T-cell proliferation (Ge *et al.*, 2005). The AGE CML in plasma was followed for six years in 1270 people aged 65 and older. In this period, 227 (22.4%) died during the period, 105 in cardiovascular disease. This mortality was significantly associated with high CML levels (Semba *et al.*, 2009).

### 22.10.4 Cancer

Individuals with high levels of oxidative stress, such as those with type 2 diabetes and significantly increased accumulation in the body of AGE/ALE, suffer a significantly increased risk of developing cancer (Abe and Yamagishi, 2008). The receptor RAGE and its multiple ligands are shown to be involved in the pathogenesis of multiple tumours: brain, breast, colon, colorectal, lung, prostate, oral squamous cell carcinoma, and ovarian cancer, as well as lymphoma and melanoma (Takada *et al.*, 2004; Genkinger *et al.*, 2006; Logsdon *et al.*, 2007). *In vitro* and animal studies, as well as preliminary clinical observations, support the view of a direct link between RAGE activation and proliferation, migration, invasion of tumour cells, and survival (Logsdon *et al.*, 2007; Abe and Yamagishi, 2008). RAGE expression is reported to be elevated in human cells with high metastatic ability and low in tumour cells with low metastatic ability (Takada *et al.*, 2004). A tumour-suppressive function of RAGE has also been reported for some distinct cell types (Gebhardt *et al.*, 2008). It is suggested that cytokines produced by cells of the innate immune system play an indispensable role in tumour-promoting inflammation, while protective anti-tumour effects derive largely from adaptive immune cells, particularly T cells (Dougan and Dranoff, 2008). An up-regulation of the gene S100P, known to be involved in the activation of RAGE, has been reported for several tumour tissues including lung, breast, pancreas, prostate, and colon (Rehbein *et al.*, 2008). The RAGE ligand sRAGE, highly expressed in healthy lung tissues especially at the site of alveolar epithelium, is significantly down-regulated in lung carcinomas (Jing *et al.*, 2010), but also in pancreatic cancer (Krechler *et al.*, 2010). The relationship between RAGE expression in surgical specimens of primary tumours and prognosis of the patient was studied recently in 216 patients with oesophageal squamous cell carcinoma (Tateno *et al.*, 2008). Those with positive RAGE expression in tumour cells exhibited a significantly better prognosis than those with negative RAGE expression (5-year survival, 52% *versus*. 32%, respectively) (Tateno *et al.*, 2008).

### 22.10.5 Cataract and other eye disorders

AGE/ALE accumulate with age in all ocular tissues including lacrimal glands, and trigger pathogenic events, especially in diabetics, in all parts of the eye (Stitt, 2005). The lens contains abundant proteins, which undergo translational modifications throughout the lifespan, contributing to ageing and cataract formation. Kynurenines are diffusible components of the lens that absorb UVA and UVB radiation, and are believed to protect the retina from light damage. However, it is also unstable under physiological conditions and undergoes deamination, its half-life being approximately 7 days. The deaminated products, known to affects lens proteins and modify specific amino acids, are believed to contribute to AGE formation, ageing of the lens, and to development of cataracts (Nagaraj *et al.*, 2010). Age-related macular degeneration (AMD) is also strongly associated with increased oxidative stress, and with increased deposition of AGE/ALE. A recent study found signs of systemic AGE accumulation in patients with AMD, implicating a role for AGE/ALE in the pathophysiology of AMD (Mulder *et al.*, 2010). The AGE CML and pentosidine are also shown to be significantly increased in AMD patients relative to healthy controls: CML (~54%), and pentosidine (~64%) ($p < 0.0001$) (Ni *et al.*, 2009). RAGE and

its ligands are also reported to be involved in retinal diseases (Barile and Schmidt, 2007) and in glaucoma (Tezel *et al.*, 2007).

### 22.10.6 Diabetes

More than 6000 publications in PubMed deal with AGE/ALE and more than half of them particularly with their role in diabetes mellitus (DM). Several excellent reviews have been published recently (Meerwaldt *et al.*, 2008; Orasanu and Plutzky, 2009; Yan *et al.*, 2009). Over-consumption of fat and carbohydrates, not only of glucose, but also of other carbohydrates such as lactose and fructose, contribute in diabetics to a significantly increased accumulation of AGE/ALE in the tissues. The consumption of high-fructose corn syrup in the United States today exceeds that of sucrose. It is ten times more capable of producing AGE/ALE, and is suggested as a major contributor, not only to obesity and accumulation of fat in the liver, but specifically to development of type 2 diabetes as well as to severe complications of both type 1 and 2 diabetes (Gaby, 2005). Chronic hyperglycaemia is suggested to alter mitochondrial function through glycation of mitochondrial proteins. A direct relationship is demonstrated between excess intracellular formation of reactive species, intracellular formation of AGE from mitochondrial proteins, and decline in mitochondrial function (Rosca *et al.*, 2005). Methylglyoxal (MGO), a highly reactive α-dicarbonil byproduct of glycolysis, which readily reacts with arginine, lysine, and sulfhydryl groups of both proteins and nucleic acids to form AGE, is significantly increased in diabetes (Ceriello, 2009). Diabetic complications such as retinopathy, nephropathy, and neuropathy are significantly associated with levels of AGE in the body. Increased levels of AGE in skin biopsies are found to be significantly associated with the outcome of micro-vascular complications (Genuth *et al.*, 2005), and closely associated with incidence and severity of diabetic complications.. Intensive control of glycaemia in insulin-dependent diabetes (IDDM) effectively delays the onset and slows down the progression of diabetic retinopathy, nephropathy, and neuropathy (DCCT, 1993). Five years of such treatment will significantly reduce various AGE/ALE in the body (30–32% lower furosine, 9% lower pentosidine, 9–13% lower CML), and increase the levels of soluble collagen (24% higher in acid-soluble collagen, and 50% higher in pepsin-soluble collagen) (Monnier *et al.*, 1999).

### 22.10.7 Endocrine disorders

Many, if not most, of the signs and symptoms of ageing, and age-associated diseases are strongly associated with multiple hormone deficiencies. Most consequences of ageing, such as excessive free radical formation, imbalance of the apoptosis systems, failure of repair systems, tissue accumulation of waste products, deficient immune system, poor gene polymorphisms, and premature telomere shortening, are all associated, if not caused, by hormone deficiencies (Hertoghe, 2005). Up-regulation of putative pathological pathways, accumulation of advanced glycation end products, activation of the renin–angiotensin system, oxidative stress, and increased expression of growth factors and cytokines are all intimately associated with ageing. However, little information is yet available about the content of AGE/ALE in endocrine organs and their influence on the body both in health or disease. With the exception of the ovaries, most of the endocrine organs – the pituitary gland, thyroids, parathyroids, adrenals, and testes – are thus far almost totally unexplored. Increased serum AGE levels and increased activation of RAGE are reported in women with polycystic ovary syndrome (PCOS) (Diamanti-Kandarakis *et al.*, 2005). A recent study reports that the content of AGE/ALE is twice as high in patients with PCOS as in healthy controls, and also strongly associated with signs of increased chronic inflammation; increases in homocysteine (Hcy), malonyldialdehyde (MDA), C-reactive protein (CRP), and with higher fasting insulin levels; and a higher homeostasis model assessment (HOMA) index (fasting glucose (mg $dl^{-1}$) x fasting insulin (mU $ml^{-1}$) x 0.055/22.5) (Kaya *et al.*, 2009). Deposition of excess collagen in PCOS tissues that induce cystogenesis are suggested,

at least in part, to be due to stimulation by AGE (Papachroni *et al.*, 2009).

### 22.10.8 Gastrointestinal disorders

Various gastrointestinal cancers and their ability ot grow and produce metastases are associated to increased levels of AGE/ALE in the body and to increased activity of RAGE. Little, however, is known about an eventual association between accumulation in the body of these molecules or activity of RAGE and common inflammatory and ulcerative conditions in the gut. The only exception seems to be a recent study, which reports that the urinary concentration of pentosidine is significantly elevated in active compared to inactive IBD, ulcerative colitis (0.12 *versus* 0.021 mg mg$^{-1}$), and Crohn's disease (0.071 *versus* 0.039 mg mg$^{-1}$) (Kato *et al.*, 2008).

### 22.10.9 Liver disorders

Patients with liver cirrhosis demonstrate much increased AGE levels, which sometimes reach almost the same extent as in patients with end-stage renal disease (Sebekova *et al.*, 2002). Serum levels of AGE (CML) are shown to be significantly affected by the stage of the disease in liver cirrhosis, and are closely associated with liver function capacity, as reported in a study 110 patients with chronic liver disease (CLD) and compared to 124 healthy controls (Yagmur *et al.*, 2006). Furthermore, the level of AGE (CML) seemed to correlate well with levels of hyaluronic acid (HA) ($r = 0.639$, $P < 0.0001$). Glyoxal-derived adducts are suggested to be increased up to no less than 15 times in both portal and hepatic venous plasma of cirrhotic patients compared to healthy controls (Ahmed *et al.*, 2005). A dramatic improvement is observed in patients after liver transplantation, although the AGE levels do not return to the levels seen in healthy controls (Sebekova *et al.*, 2002). Animal studies suggest that blockage of RAGE is highly protective against hepatocellular necrosis and cell death, and to significantly increase the rate of survival (Zeng *et al.*, 2004; Ekong *et al.*, 2006). A significant increase in glutathione and pro-regenerative cytokines TNF-α and IL-6 are observed, in addition to decreased hepatic necrosis and increased survival (Ekong *et al.*, 2006). Much remains to be done to define the role of AGE/ALE and RAGE in the progression of non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH) and cirrhosis. However, recent observations of serum glyceraldehyde-derived AGE levels (U ml$^{-1}$) being significantly elevated in NASH patients (9.78) compared to those with simple steatosis (7. $P = 0.018$) and healthy controls (6.96, $P = 0.003$) are of significant interest (Hyogo *et al.*, 2007). These authors also observed an inverse correlation to the level of adiponectin, an adipocytokine with insulin-sensitizing and anti-inflammatory properties. Immunohistochemistry of glyceraldehyde-derived AGE also showed increased staining in the livers of NASH patients.

### 22.10.10 Lung disorders

A variety of airway diseases such as asthma, acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD), cystic fibrosis, and idiopathic pulmonary fibrosis are all characterized by lack of homeostasis in the oxidant/antioxidant balance. Interaction of AGE/ALE and RAGE are well known to play a large, if not dominating, role in the depletion of antioxidants, particularly reducted glutathione (GSH) in lung epithelial lining and to play a key role in the pathogenesis of these disorders (Foell *et al.*, 2003; Rahman *et al.*, 2006a).

### 22.10.11 Rheumatoid arthritis and other skeletomuscular disorders

Among the highest levels of AGE in the body, and the strongest expression of RAGE, are found in inflamed tissues characterized for slow turnover, such as tendons, bone, cartilage, skin, and amyloid plaques. These changes are associated with a slight change in colour towards yellow-brown and and an increased fluorescence, all associated with

increased expression of pro-inflammatory cytokines and matrix metalloproteinases (MMP), especially MMP-1 and MMP-9. These manifestations are regarded as being responsible for the observed increased tissue stiffness and brittleness in structures such as intervertebral discs, bones, tendons, cartilages, synovial membranes, and skeletal muscles, and are regarded as major pathogenic factors behind diseases such as osteoarthritis (DeGroot, 2004; Steenvoorden, 2006), rupture of inter-vertebral discs (Hormel and Eyre 1991), rupture of Achilles tendons (Reddy, 2004), menisci, and also in rheumatoid diseases (Hein *et al.*, 2005; Sunahori, *et al.*, 2006), such as rheumatoid arthritis (Matsumoto *et al.*, 2007) and fibromyalgia (Hein and Franke, 2002; Rüster *et al.*, 2005). A significant increase in glycation of myosin occurs with age (Ramamurthy *et al.*, 2001), and is most likely to contribute to age-associated muscular disorders. High levels of AGE/ALE in the body are also reported in patients with osteoporosis; significantly elevated levels of pentosidine and CML in serum (Hein *et al.*, 2003) and significantly increased pentosidine in cortical bone (Odetti *et al.*, 2005) being observed. It has also been observed that the remodelling of senescent bone is impaired by AGE, with both stimulation of bone-resorbing cytokines and enhancement of bone resorption by osteoclasts being observed (Miyata *et al.*, 1996). A recent American study reports a significantly reduced bone density in older women consuming > 3 cola drinks per week when compared to matched controls consuming similar amounts of other carbonated soft drinks (Tucker *et al.*, 2006). This information is especially interesting when one considers that cola drinks, much in contrast to other soft drinks, are considered rich in AGE. A recent *in vitro* study reports profound effects by cola-derived AGE: activation of platelets, an up to 7.1-fold increase in CD62 expression, and an increase of up to 2.2-fold in CD63 at the platelet surface membrane, also accompanied by increases in RAGE expression (Gawlowski *et al.*, 2009).

The common belief that bovine milk prevents osteoporosis is today much questioned. Instead, increasing documentation suggests that it has quite the opposite effects, and that negative interactions of RAGE and AGE/ALE play a larger role in the pathogenesis of osteoporosis than lack of minerals.

### 22.10.12 Skin and oral cavity issues

The skin is one of, if not the largest, organ in the body. The health condition of the skin has a similar ability to the gingiva in the mouth, to reflect the total health of the body. Skin autofluorescence seems to be a good measure of cumulative metabolic stress and accumulation of advanced glycation end products in the body (Meerwaldt *et al.*, 2005a,b). Accumulation of AGE/ALE in the skin relates to the content of these proteins in the body, and so is an expression of the risk of developing chronic diseases, in particular coronary heart disease. A recent study found a significant correlation between coronary calcifications and AGE/ALE in the body measured as skin fluorescence, suggesting such measurements could serve both as a marker of risk but also as a measurement of therapeutic success when patients are treated (Conway *et al.*, 2010). Skin autofluorescence is especially suggested to be a method for prediction of the risk for progression of diabetic complications such as angiopathy, nephropathy, and retinopathy, and the severity of disease and mortality in haemodialysis patients (Meerwaldt *et al.*, 2005b).

The skin has a high density of RAGE receptors. AGE/ALE are known to accumulate in dermal elastine and in collagens, and to interact with dermal fibroblasts, inhibiting their proliferation capacity. A tenfold reduction in proliferation rate is described to occur normally in humans from the second to seventh decade (Stamatas *et al.*, 2006), and is suggested as explaining the reduced healing capacity of age-related wounds, and especially chronic wounds, such as those on the diabetic foot. RAGE and AGE/ALE-induced apoptosis and enhanced loss of fibroblasts and osteoblasts are also regarded as major pathogenic factors in periodontal pathology, especially in chronic periodontitis (Holla *et al.*, 2001). A 50% increase is observed compared with controls in RAGE mRNA in gingiva of diabetic patients ($p<0.05$) (Katz *et al.*, 2005).

### 22.10.13 Urogenital disorders

Nephropathy is today more common than ever before and continues to increase, much in parallel to the increase in diabetes. It is the single most important cause of end-stage renal failure in the western world (Ostergaard *et al.*, 2005). Consequently it s receives great interest from scientists. Today no less than 1000 papers in PubMed deal with RAGE and AGE/ALE in renal diseases. Diabetic nephropathy today affects 15–25% of patients with type 1 diabetes, and as much as 30–40% of patients with type 2 diabetes. The kidney appears as both culprit and target of AGE/ALE, and it is well documented that RAGE is significantly activated, and advanced AGE/ALE markedly elevated in renal failure patients. Patients with mild chronic uremic renal failure are reported to have plasma glycation-free adduct concentrations increased up to fivefold, patients with end-stage renal disease as much as 18-fold when on peritoneal dialysis, and up to 40-fold on haemodialysis (Agalou *et al.*, 2005). A decrease in renal function and reduced clearance is observed much in parallel to increases in circulating AGE levels. AGE are involved in the structural changes observed in progressing nephropathies such as glomerulosclerosis, interstitial fibrosis, and tubular atrophy (Bohlender *et al.*, 2005). For detailed information, see recent excellent reviews (Vlassara *et al.*, 2009; Daroux *et al.*, 2010; Schepers *et al.*, 2010). Kidney transplantation is reported to improve but does not fully correct the increased AGE/ALE levels in previously dialysed patients.

## 22.11 Foods Rich in AGE/ALE

It is a most interesting observation that increased accumulation of AGE/ALE in endothelial cells, and most likely also in some other tissues, can be significantly avoided or reduced by control of intake of foods known to contain these substances in large amounts. Thus far the information regarding content of AGE/ALE in foods is rather incomplete. Leading universities around the world are building institutions for studies of nutragenomics (how various food ingredients affect our health). However, from existing information it is clear that dysfunctioning proteins are especially rich in foods which have been subjected to industrial processing. The foods with the highest AGE content were animal-derived products exposed to high, dry heat such as broiling, frying, and grilling. A detailed description of the database can be found in Goldberg *et al.* (2004). A brief summary is provided below.

- *Heated dairy products:* powdered milk (ice cream, baby and clinical nutrition formulas) cheese, especially when heated. High in pizza, tacos, nachos, salads, fast food, sandwiches, sauces, and brown cheeses.
- *Heated grain products:* bread (e.g. toasted bread, bread crusts, and crispbreads).
- *Heated meat, poultry, and fish:* especially bacon, sausages, fried, and barbecued meat. The content of AGE/ALE increases as one goes from boiling to oven frying: boiling (1000 kU/serving) < roasting (4300 kU) < broiling (5250 kU) < deep frying (6700 kU) < oven frying (9000 kU/serving) (Goldberg *et al.*, 2004).
- *Other heated foods:* egg yolk powder, lecithin powder, coffee (especially dark roasted), hard-cured teas, roasted and salted peanuts, dark and sugar-rich alcoholic beverages, broth, Chinese soy, balsamic vinegar, cola drinks, etc.

A recent study adds further and important information about dietary AGE (Uribarri *et al.*, 2010). It should be observed that lean red meats and poultry contain high levels of dietary AGE. Even when cooked under dry heat, the explanation is that among the intracellular components of lean muscle there are highly reactive amino lipids, as well as reducing sugars such as fructose or glucose-6-phosphate. In the presence of heat, this combination rapidly accelerates new AGE formation (Uribarri *et al.*, 2010). The highest AGE levels are observed in beef and cheeses followed by poultry, pork, fish, and eggs, while lamb ranked relatively low in AGE, at least when compared to other meats. Cheeses, butter, and different types of oils are AGE-rich, even in uncooked forms. High-fat spreads, including butter,

cream cheese, margarine, and mayonnaise, are also among the foods containing the highest AGE, followed by oils and nuts. It should especially be observed that olive oil, for example, contains large amounts of AGE when heated to 100°C for 5 min (Uribarri *et al.*, 2010). Carbohydrate-rich foods such as vegetables, fruits, and whole grains contain relatively few AGE, even after cooking (Uribarri *et al.*, 2010).

## 22.12 Prevention and Treatment of AGE/ALE Accumulation

### 22.12.1 Changing food preparation habits

It is clear that significant benefits will be obtained by reducing the intake of cheese, meats, powdered milk, other processed foods such as heated oils, and also of bread; and instead increase the consumption of vegetables and fruits, especially when raw. These recommendations are in line with the policy of various expert organizations with the aim to reduce chronic diseases such as cancer, heart diseases, and hypertension (cancer: American Cancer Society, 2006; heart: Lichtenstein *et al.*, 2006; hypertension: US Department of Health and Human Services *et al.*, 2006). AGE formation in food is reduced when cooking on surfaces that provide no direct contact with metal; when foods are immersed in acid solutions such as tomato sauce and ketchup; and when there is contact with aminoguanidine, a known inhibitor of AGE formation (He *et al.*, personal communication, 2010). Eating foods raw or prepared at a low temperature (below 80°C), steam cooking or boiling, and minimal cooking are preferred over frying, grilling, and microwaving, and also to roasting and salting. Recent information seems especially to warn against microwaving food, as this treatment dramatically accelerates the rate of AGE production (Visentin *et al.*, 2010). A trial designed to compare the potential metabolic effects of two different diets, one based on mild steam cooking and another based on high-temperature cooking was recently reported (Birlouez-Aragon *et al.*, 2010). A randomized crossover study assigned 62 volunteers (university students) to each of the two diets for four weeks. Consuming the steamed-cooked diet for 1 month induced significantly improved insulin sensitivity and also increased plasma levels of omega-3 fatty acids (217%, $p = 0.002$), vitamin C (213%, $p = 0.0001$), and vitamin E (28%, $p = 0.01$), in comparison to the high temperature diet. Furthermore, reduced concentrations of plasma cholesterol (5%, $p = 0.01$) and triglycerides (9%, $p = 0.01$) were also reported.

A challenge for the future in the Western world is to find techniques to produce bread at 100°C or below as the Chinese have done for centuries. Marinating for some hours at room temperature with ingredients such as antioxidant-rich herbs, garlic, tea, red wine, onions, olive oil, and beer are also known to significantly reduce the development of AGE/ALE, and this was also recently demonstrated for heterocylic aromatic amines (Melo *et al.*, 2008). Reduction in total intake of proteins (Uribarri and Tuttle, 2006), and most likely a particular reduction in methionine and other sulphur-containing amino acids, are additional issues of relevance (McCarty *et al.*, 2009).

### 22.12.2 Energy restriction

Significant reduction in body content of AGE/ALE in comparison to controls (eating standard Western food) is observed in individuals, who for > 2 years practise what is called caloric restriction (CR). They eat only two thirds of what they would like to, and this is accompanied by significant health advantages compared to matched controls: lower blood pressure (102/61±7 versus 131/83 mm Hg), and lower levels of markers of inflammation such as CRP (0.3 versus 1.9 mg l), TNF-α (0.8 versus 1.5 pg $ml^{-1}$), and TGF-β (29.4 versus 35.4 ng $ml^{-1}$) (Meyer *et al.*, 2006). Elevated RAGE and low sRAGE is reported in patients with active rheumatoid arthritis (RA), but patients with RA practising CR for about 2 months demonstrated not only lower levels of pentosidine (an often-measured AGE) in urine, but also lower disease activity (Iwashige *et al.*, 2004). Thirty-seven obese individuals (mean BMI of 28.3 ± 3.2) were treated with calorie restriction for 8 weeks. Reduction occurred in BMI (6.3%, $p < 0.001$), waist

circumference (5.7%, $p < 0.002$), triglycerides (11.9 % ($p < 0.002$), and AGE (7.21%, $p < 0.001$) (Gugliucci *et al.*, 2009). FEV1, an expression of respiratory capacity, almost doubled.

### 22.12.3 Antioxidants and vitamins

Provision of vitamins such as A, B (especially $B_6$ and $B_{12)}$, C, D, E, and K, as well as glutathione and folic acid, is often emphasized. Many plant antioxidants, particularly those collectively defined as polyphenols, have documented oxidation-quenching properties up to ten times more powerful than conventional vitamins. They have also been shown to have great chemo-preventive properties, a marked ability to prevent accumulation in the body of AGE/ALE, and significant capacities to reduce inflammation in the body and to prevent reduction in organ function and premature ageing (Delmas *et al.*, 2005; Osawa and Kato, 2005; Bengmark, 2006; Rahman *et al.*, 2006b; Sun *et al.*, 2010). Such plant antioxidants exist in nature in many thousands – most probably hundreds of thousands – of different compounds. More than 4000 flavonoids alone have been identified, and almost 1000 carotenoids. Here are the most studied: isothiocyanates in cruciferous vegetables; anthocyanins and hydroxycinnamic acids in cherries; epigallocatechin-3-gallate (EGCG) in green tea; chlorogenic acid and caffeic acid in fresh coffee beans and also in fresh tobacco leaves; capsaicin in hot chilli peppers; chalcones in apples; euginol in cloves; gallic acid in rhubarb; hisperitin and naringenin in citrus fruits; kaempferol in white cabbage; myricetin in berries; rutin and quercetin in apples and onions; resveratrol and other procyanidin dimers in red wine and virgin peanuts; various curcumenoids, the main yellow pigments in turmeric curry foods; and daidzein and genistein from the soy bean.

### 22.12.4 Supplementing histidine, taurine, carnitine, or carnosine

Supplementing the diet with histidine, taurine, carnitine, or carnosine has also been reported to assist in protecting the body from AGE/ALE (Nandhini *et al.*, 2004, 2005). No vegetarian food contains taurine, with the exception of certain algae. This important amino acid is only obtained from eating animal-derived foods – meat, poultry, and fish.

### 22.12.5 Pharmaceuticals

Several pharmaceuticals, especially those used for treatment of diabetes, are reported to reduce the content of AGE/ALE in the body, at least in short-lived tissues; that is, those with high turnover.

## 22.13 Pro- and Synbiotics

Probiotics and synbiotics have a dual role in reduction of dietary AGE/ALE as they both metabolize these substances (Erbersdobler *et al.*, 1970; Finot and Magnenat, 1981; Faist and Erbersdobler, 2001; Faist *et al.*, 2001, Wiame *et al.*, 2002) and also release important vitamins and antioxidants with documented preventive effects against AGE and ALE. A rich intestinal flora is regarded as necessary for the release and absorption of various important antioxidants. However, the increased intake of refined food and deficient intake of fresh fruits and vegetables among Westerners has led to a significant reduction in both density and diversity of the flora. This reduction is especially pronounced for strong fibre-fermenting lactic acid bacteria (LAB) such as *Lactobacillus plantarum* and *Lb. paracasei*. Seventy-five per cent of omnivorous Americans and 25% of vegetarians in the United States lack *Lb. plantarum* (Finegold *et al.*, 1983). A more recent Scandinavian study found *Lb. plantarum* in only 52% and *Lb. paracasei* in only 17% of healthy individuals (Ahrné *et al.*, 1998). This information is particularly interesting, as *Lb. plantarum* and *Lb. paracasei* belong to the small group of intestinal bacteria with ability to break down semi-resistant fibres such as inulin (Müller and Lier, 1994), reduce inflammation, reduce infection, and eliminate pathogenic bacteria such as *Clostridium difficile* (Naaber *et al.*, 2004). Some specific LAB might well have the ability to

eliminate AGE/ALE from foods, in a way that is very similar to that demonstrated for gluten (di Cagno *et al.*, 2005) and heterocyclic amines (Tavan *et al.*, 2002). *In vitro* studies have shown that fructoselysine, the dominating AGE in heated milk, can be effectively eliminated when incubated with fresh intestinal flora (Erbersdobler *et al.*, 1970).

## 22.14 Conclusions

There is growing consensus that the dietary intake or endogenous production of AGE/ALE is associated with a diverse range of disorders, although the underlying mechanisms remain largely obscure. This chapter has focused on the dietary sources of AGE/ALE and on the effects of heat treatment in the production of these dysfunctional adducts during the processing of foods. The accumulation of AGE has been documented in conditions such as Alzheimer's disease, cardiovascular disease, cancer, diabetes, lung disease, liver disease, and other disorders, and this research evidence has also been reviewed in this chapter.

It is increasingly clear that the intestinal microflora and its more than 2 million metagenes play a key role in health and disease. Western lifestyle is clearly associated with a deranged microbionta, with reduced diversity and an increased quotient between gram-negative and gram-positive bacteria, which in association with reduced barrier function seems to contribute to the observed elevated systemic inflammation. Patients with metabolic derangements such as obesity and with chronic diseases are known to have increased blood levels of endotoxin, a product of gram-negative bacteria. Recent *in vitro* observations suggest that human AGE-modified albumin and lipopolysaccharide (LPS) exhibit a synergistic effect on proinflammatory cytokine/chemokine interleukin-6, interleukin-8, and monochemoattractant protein-1 production in human endothelial cells (Liu *et al.*, 2009). A link exists between fat intake and accumulation of endotoxin in the blood (endotoxaemia), and recent studies demonstrate that intake of emulsified fat in particular (water-in-oil emulsions such as butter; free oil or dispersed fat inclusions in cheeses, cookies, ice cream, and dressings), which is known to affect the kinetics of lipid absorption, increases both endoxaemia and inflammation (Laugerette *et al.*, 2010).

It is now well documented in the literature that a healthy lifestyle has profound effects on health and well-being. Control of what we eat is an important component within such a programme. Studies suggest reductions of as much as 83% in coronary heart disease (Stampfer *et al.*, 2000), 91% in diabetes (Hu *et al.*, 2001), and 71% in colon cancer (Platz *et al.*, 2000) in patients adhering to a 'healthy lifestyle' (such as no use of tobacco, moderate use of alcohol, regular physical exercise, and controlled food intake). To these four factors must be added control of stress. Numerous studies demonstrate that both physical and mental stress increases the degree of inflammation in the body and activates RAGE (Kjaer, 2004; Bierhaus *et al.*, 2006; Chida *et al.*, 2006). Control of intake and endogenously produced AGE/ALE, will, together with restrictions on the intake of fat and carbohydrate-rich foods, significantly improve health and well-being. However, only a fraction of consumers are willing to consider this option. A study in the United States (Reeves and Rafferty, 2005), suggests that only about 3% adhere to the principles advocated above.

## References

Abe, R. and Yamagishi, S. (2008) AGE-RAGE system and carcinogenesis. *Current Pharmaceutical Design* 14, 940–945.

Agalou, S., Ahmed, N. Babaei-Jadidi, R., *et al.* (2005) Profound mishandling of protein glycation degradation products in uremia and dialysis. *Journal of the American Society of Nephrology* 16, 1471–1485.

Ahmed, N., Lüthen, R., Häussinger, D., *et al.* (2005) Increased protein glycation in cirrhosis and therapeutic strategies to prevent it. *Annals of the New York Academy of Sciences* 1043, 718–724.

Ahrné, S., Nobaek, S., Jeppsson, B., *et al.* (1998) The normal Lactobacillus flora of healthy human rectal and oral mucosa. *Journal of Applied Microbiology* 85, 88–94.
Allen, T.J. and Jandeleit-Dahm, K.A. (2005) Preventing atherosclerosis with angiotensin-converting enzyme inhibitors: emphasis on diabetic atherosclerosis. *Current Drug Targets - Cardiovascular & Hematological Disorders* 5, 503–512.
Alverdy. J.C., Laughlin, R.S. and Wu, L. (2003) Influence of the critically ill state on host-pathogen interactions within the intestine: gut-derived sepsis redefined. *Critical Care Medicine* 31, 598–607.
American Cancer Society (2006) Choices for good health: American Cancer Society guidelines for nutrition and physical activity for cancer prevention. *CA – A Cancer Journal for Clinicians* 56, 310–312.
Aronsen, D. (2003) Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. *Journal of Hypertension* 21, 3–12.
Artaud-Wild, S.M., Connor, S.L., Sexton, G. and Connor, W.E. (1993) Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. A paradox. *Circulation* 88, 2771–2779.
Baptista, J.A.B. and Carvalho, R.C.B. (2004) Indirect determination of Amadori compounds in milk-based products by HPLC/ELSD/UV as an index of protein detorioration. *Food Research International* 37, 739–747.
Barile, G.R. and Schmidt, A.M. (2007) RAGE and its ligands in retinal disease. *Current Molecular Medicine* 7, 758–765.
Barnard, N.D., Cohen, J., Jenkins, D.J., *et al.* (2009) A low-fat vegan diet and a conventional diabetes diet in the treatment of type 2 diabetes: a randomized, controlled, 74-wk clinical trial. *American Journal of Clinical Nutrition* 89, 1588S–1596S.
Bengmark, S. (2006) Curcumin: an atoxic antioxidant and natural NF-κB, COX-2, LOX and iNOS inhibitor – a shield against acute and chronic diseases. *Journal of Parenteral and Enteral Nutrition* 30, 45–51.
Bierhaus, A., Schiekofer, S., Schwaninger, M., *et al.* (2001) Diabetes-associated sustained activation of the transcription factor nuclear factor –κB. *Diabetes* 50, 2792–2808.
Bierhaus, A., Humpert, P.M., Stern, D.M., *et al.* (2005a) Advanced glycation end product receptor-mediated cellular dysfunction. *Annals of the New York Academy of Sciences* 1043, 676–680.
Bierhaus, A., Humpert, P.M., Morcos, M., *et al.* (2005b) Understanding RAGE, the receptor for advanced glycation end products. *Journal of Molecular Medicine* 83, 876–886.
Bierhaus, A., Humpert, P.M. and Nawroth, P.P. (2006) Linking stress to inflammation. *Anesthesiology Clinics* 24, 325–340.
Birlouez-Aragon, I. and Tessier, F.J. (2003) Antioxidant vitamins and degenerative pathologies. A review of vitamin C. *Journal of Nutrition, Health & Aging* 7, 103–109.
Birlouez-Aragon, I., Saavedra, G., Tessier, F.J., Galinier, A., Ait-Ameur, L., Lacoste, F., Niamba, C.N., *et al.* (2010) A diet based on high-heat-treated foods promotes risk factors for diabetes mellitus and cardiovascular diseases. *American Journal of Clinical Nutrition* 91, 1220–1226.
Bohlender, J.M. Franke, S., Stein, G. and Wolf, G. (2005) Advanced glycation end products and the kidney. *American Journal of Physiology - Renal Physiology* 289, F645–659.
Boratynski, J. and Gorski. A. (2002) BSE: a consequence of cattle feeding with glycated molecules host-unknown? *Medical Hypotheses* 58, 276–278.
Brady, J. and Hoggan, R. (2002) *Dangerous grains*. Avery-Penguin Putnam, New York.
Brandtzaeg, P., Halstensen, T.S., Kett, K., *et al.* (1989) Immunobiology and immunopathology of human gut mucosa: Humoral immunity and intraepithelial lymphocytes. *Gastroenterology* 97, 1562–1584.
Burns, M., Gaynor, K. and Olm, V. (2003) Presenilin redistribution associated with aberrant cholesterol transport enhances beta-amyloid production in vivo. *Journal of Neuroscience* 23, 5645–5649.
Ceriello, A. (2009) Hypothesis: the "metabolic memory", the new challenge of diabetes. *Diabetes Research and Clinical Practice* 86, S2–S6.
Chida, Y., Sudo, N. and Kubo, C. (2006) Does stress exacerbate liver diseases? *Journal of Gastroenterology & Hepatology* 21, 202–208.
Conway, B., Edmundowicz, D., Matter, N., *et al.* (2010) Skin fluorescence correlates strongly with coronary artery calcification severity in type 1 diabetes. *Diabetes Technology & Therapeutics* 12, 339–345.
Cooper, E.V. (1946) Gas gangrene following injection of adrenaline. *Lancet* 247, 459–461.
Carroll, K.K. (1975) Experimental evidence of dietary factors and hormone-dependent cancers. *Cancer Research* 35, 3374–3383.
Castellani, R., Smith, M.A., Richey, P.J. and Perry, G. (1996) Glycoxidation and oxidative stress in Parkinson disease and diffuse Lewy body disease. *Brain Research* 737, 195–200.

Choi, Y.G., Kim, J.I., Jeon, Y.C., *et al.* (2004) Nonenzymatic glycation at the N terminus of pathogenic prion protein in transmissible spongiform encephalopathies. *Journal of Biological Chemistry* 279, 30402–30409.

Chou, S.M., Wang, H.S., Taniguchi, A. and Bacula, R. (1998) Advanced glycation end products in neurafilament conglomeration of motorneurons in familial and sporadic amyotrophic lateral sclerosis. *Molecular Medicine* 4, 324–332.

Chung, S.Y. and Champagne, E.T. (2001) Association of end-product adducts with increased IgE binding of roasted peanuts. *Journal of Agricultural and Food Chemistry* 49, 3911–3916.

Dahl-Jorgensen, K., Joner, G. and Hanssen, K.F. (1991) Relationship between cows' milk consumption and incidence of IDDM in childhood. *Diabetes Care* 14, 1081–1083.

Dalfo, E., Portero-Otin, M., Ayala, V., *et al.* (2005) Evidence of oxidative stress in the neocortex in incidental Lewy body disease. *Journal of Neuropathology & Experimental Neurology* 64, 816–830.

Daroux, M., Prévost, G., Maillard-Lefebvre, H., *et al.* (2010) Advanced glycation end-products: implications for diabetic and non-diabetic nephropathies. *Diabetes & Metabolism* 36, 1–10.

Davis, P.J., Smales, C.M. and James, D.C. (2001) How can thermal processing modify the antigenicity of proteins? *Allergy* 56, 56–60.

DCCT (Diabetes Control and Complications Trial) (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *New England Journal of Medicine* 329, 977–986.

DeGroot, J. (2004) The AGE of the matrix: chemistry, consequence and cure. *Current Opinion in Pharmacology* 4, 301–305.

De Leeuw, K., Kallenberg, C. and Bijl, M. (2005) Accelerated atherosclerosis in patients with systemic autoimmune diseases. *Annals of the New York Academy of Sciences* 1051, 362–371.

Delmas, D., Jannin, B. and Latruffe, N. (2005) Resveratrol: preventing properties against vascular alterations and ageing. *Molecular Nutrition & Food Research* 49, 377–395.

Diamanti-Kandarakis, E., Piperi, C., Kalofoutis, A. and Creatsas, G. (2005) Increased levels of serum advanced glycation end-products in women with polycystic ovary syndrome. *Clinical Endocrinology* 62, 37–43.

di Cagno, R., de Angelis, M., Alfonsi, G., *et al.* (2005) Pasta made from durum wheat semolina fermented with selected lactobacilli as a tool for a potential decrease of the gluten intolerance. *Journal of Agricultural and Food Chemistry* 53, 4393–4402.

Dougan, M. and Dranoff, G.(2008) Inciting inflammation: the RAGE about tumor promotion. *Journal of Experimental Medicine* 205, 267–270.

Ekong, U., Zeng, S., Dun, H., *et al.* (2006) Blockade of the receptor for advanced glycation end products attenuates acetaminophen-induced hepatotoxicity in mice. *Journal of Gastroenterology & Hepatology* 21, 682–688.

Erbersdobler, H., Gunsser, I. and Weber, G. (1970) Abbau von Fructoselysine durch die Darmflora. *Zentralblatt für Veterinärmedizin* A17, 573–575.

Faist, V. and Erbersdobler, H.F. (2001). Metabolic transit and in vivo effects of melanoidins and precursor compounds deriving from the Maillard reaction. *Annals of Nutrition and Metabolism* 45, 1–12.

Faist, V., Wenzel, E. and Erbersdobler, H.F. (2000) *In vitro* and *in vivo* studies on the metabolic transit of Nε-carboxymethyllysine. *Czech Journal of Food Sciences* 18, 116–119.

Ferretti, G., Bacchetti, T. and Negre-Salvayre, A. (2006) Structural modifications of HDL and functional consequences. *Atherosclerosis* 184, 1–7.

Finegold, S.M., Sutter, V.L. and Mathisen, G.E. (1983) Normal indigenous intestinal flora. In: Hentges, D.J. (ed.) *Human Intestinal Microflora in Health and Disease*. Academic Press, London, pp. 3–31.

Finot, P.A. and Magnenat, E. (1981) Metabolic transit of early and advanced Maillard products. *Progress in Food & Nutrition Science* 5, 193–207.

Flyvbjerg, A., Khatir, D.S., Jensen. L.J., *et al.* (2004) The involvement of growth hormone (GH), insulin-like growth factors (IGFs) and vascular endothelial growth factor (VEGF) in diabetic kidney disease. *Current Pharmaceutical Design* 10, 3385–3394.

Foell, D., Seeliger, S., Vogl, T., *et al.* (2003) Expression of S100A12 (EN-RAGE) in cystic fibrosis. *Thorax* 58, 613–617.

Franck, P., Moneret Vautrin, D.A., Dousset, B., *et al.* (2002) The allergenicity of soybean-based products is modified by food technologies. *International Archives of Allergy and Applied Immunology* 128, 212–219.

Frey, J. (2002) Bovine spongiform encephalopathy: are the cows mad or full of carbohydrate. *Clinical Chemistry and Laboratory Medicine* 40, 101–103.

Gaby, A.R. (2005) Adverse effects of dietary fructose. *Alternative Medicine Review* 10, 294–306.
Ganmaa, D., Li, X.M. and Wang, J. (2002) Incidence and mortality of testicular and prostatic cancers in relation to world dietary practices. *International Journal of Cancer* 98, 262–267.
Ganmaa, D., Li, X.M., Qin, L.Q., *et al.* (2003) The experience of Japan as a clue to the etiology of testicular and prostatic cancers. *Medical Hypotheses* 60, 724–730.
Gawlowski, T., Stratmann, B. and Ruetter, R. (2009) Advanced glycation end products strongly activate platelets. *European Journal of Nutrition* 48, 475–481.
Ge, J., Jia, Q., Liang, C., *et al.* (2005) Advanced glycosylation end products might promote atherosclerosis through inducing the immune maturation of dendritic cells. *Arteriosclerosis, Thrombosis, and Vascular Biology* 25, 2157–2163.
Gebhardt, C., Riehl, A., Durchdewald, M., Németh, J., Fürstenberger, G., Müller-Decker, K., Enk, A., *et al.* (2008) RAGE signaling sustains inflammation and promote tumor development. *Journal of Experimental Medicine* 205, 275–285.
Genkinger, J.M., Hunter, D.J., Spiegelman, D., *et al.* (2006) Dairy products and ovarian cancer: a pooled analysis of 12 cohort studies. *Cancer Epidemiology, Biomarkers & Prevention* 15, 364–372.
Genuth, S., Sun, W., Cleary, P., *et al.* (2005) Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. *Diabetes* 54, 3103–3111.
Goldberg, T., Cai, W., Peppa, M., *et al.* (2004) Advanced glycoxidation end products in commonly consumed foods. *Journal of the American Dietetic Association* 104, 1287–1291.
Gomes, R., Sousa Silva, M., Quintas, A., *et al.* (2005) Argpyrimidine, a methylglyoxal-derived advanced glycation end-product in familial amyloidotic polyneuropathy. *Biochemical Journal* 385, 339–345.
Gugliucci, A., Kotani, K., Taing, J., *et al.* (2009), Short-term low calorie diet intervention reduces serum advanced glycation end products in healthy overweight or obese adults. *Annals of Nutrition* and *Metabolism* 54, 197–201.
Hein, G. and Franke, S. (2002) Are advanced glycation end-product-modified proteins of pathogenetic importance in fibromyalgia? *Rheumatology* 41, 1163–1167.
Hein, G., Wiegand, R., Lehmann, G., *et al.* (2003) Advanced glycation end-products pentosidine and N epsilon-carboxymethyllysine are elevated in serum of patients with osteoporosis. *Rheumatology* 42, 1242–1246.
Hein, G.E., Kohler, M., Oelzner, P., *et al.* (2005) The advanced glycation end product pentosidine correlates to IL-6 and other relevant inflammatory markers in rheumatoid arthritis. *Rheumatology International* 26, 137–141.
Hertoghe, T. (2005). The "multiple hormone deficiency" theory of aging: is human senescence caused mainly by multiple hormone deficiencies? *Annals of the New York Academy of Sciences* 1057, 448–465.
Holla, L.I., Kankova, K., Fassmann, A., *et al.* (2001) Distribution of the receptor for advanced glycation end products gene polymorphisms in patients with chronic periodontitis: a preliminary study. *Journal of Periodontology* 72, 1742–1746.
Hormel, S.E. and Eyre, D.R. (1991) Collagen in the ageing human intervertebral disc: an increase in covalently bound fluorophores and chromophores. *Biochimica et Biophysica Acta* 1078, 243–250.
Hostettler-Allen, R.L., Tappy, L. and Blum, J.W. (1994) Insulin resistance, hyperglycemia, and glucosuria in intensively milk-fed calves. *Journal of Animal Science* 72, 160–173.
Hu, F.B., Manson, J.E., Stampfer, M.J., *et al.* (2001) Diet, lifestyle and the risk of type 2 diabetes mellitus in women. *New England Journal of Medicine* 345, 790–797.
Hyogo, H., Yamagishi, S., Iwamoto. K., *et al.* (2007) Elevated levels of serum advanced glycation end products in patients with non-alcoholic steatohepatitis. *Journal of Gastroenterology & Hepatology* 22, 1112–1119.
Iwashige, K., Kouda, K., Kouda, M., *et al.* (2004) Calorie restricted diet and urinary pentosidine in patients with rheumatoid arthritis. *Journal of Physiological Anthropology and Applied Human Science* 23, 19–24.
Jing, R., Cui, M., Wang, J. and Wang, H. (2010) Receptor for advanced glycation end products (RAGE) soluble form (sRAGE): a new biomarker for lung cancer. *Neoplasma* 57, 55–61
Kalousova, M., Zak, A. and Soukupova, J. (2005) Advanced glycation and oxidation products in patients with atherosclerosis. *Cas Lek Cesk* 144, 385–390 [in Czech].
Kato, S., Itoh, K., Ochiai. M., *et al.* (2008) Increased pentosidine, an advanced glycation end-product, in urine and tissue reflects disease activity in inflammatory bowel diseases. *Journal of Gastroenterology & Hepatology* 2, S140–S145.

Katz, J., Bhattacharyya, I., Farkhondeh-Kish, F., *et al.* (2005) Expression of the receptor of advanced glycation end products in gingival tissues of type 2 diabetes patients with chronic periodontal disease: a study utilizing immunohistochemistry and RT-PCR. *Journal of Clinical Periodontology* 32, 40–44.

Kaufmann, E., Boehm, B.O. and Sussmuth, S.D. (2004) The advanced glycation end-product N epsilon-(carboxymethyl)lysine level is elevated in cerebrospinal fluid of patients with amyotrophic lateral sclerosis. *Neuroscience Letters* 371, 226–229.

Kaya, C., Erkan, A.F., Cengiz, S.D., *et al.* (2009) Advanced oxidation protein products are increased in women with polycystic ovary syndrome: relationship with traditional and nontraditional cardiovascular risk factors in patients with polycystic ovary syndrome. *Fertility and Sterility* 92, 1372–1377.

Kikuchi, S., Shinpo, K., Ogata, A., *et al.* (2002) Detection of N-(carboxymethyl)lysine (CML) and non-CML advanced glycation end products in the anterior horn of amyotrophic lateral sclerosis spinal cord. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders* 3, 63–68.

Kinney, K.S., Austin, C.E., Morton, D.S. and Sonnenfeld, G. (2000) Norepinephrine as a growth stimulating factor in bacteria–mechanistic studies. *Life Science* 67, 3075–3085.

Kjaer, M. (2004) Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiological Reviews* 84, 649–698.

Koyama, H., Shoji, T., Yokoyama, H., *et al.* (2005) Plasma level of endogenous secretory RAGE is associated with components of the metabolic syndrome and atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology* 25, 2587–2593.

Krechler, T., Jáchymová, M. and Mestek, O. (2010) Soluble receptor for advanced glycation end-products (RAGE) and polymorphisms of RAGE and glyoxalase I genes in patients with pancreas cancer. *Clinical Biochemistry* 43, 10–11.

Kurien, B.T., Hensley, K., Bachmann, M. and Scofield, R.H. (2006) Oxidatively modified autoantigens in autoimmune diseases. *Free Radical Biology & Medicine* 41, 549–556.

Lalla, E., Lamster, I.B., Stern, D.M. and Schmidt, A.M. (2001) Receptor for advanced glycation end products, inflammation, and accelerated periodontal disease in diabetes: mechanisms and insights into therapeutic modalities. *Annals of Periodontology* 6, 113–118.

Larsson, S.C., Bergkvist, L. and Wolk, A. (2004) Milk and lactose intakes and ovarian cancer risk in the Swedish Mammography Cohort. *American Journal of Clinical Nutrition* 80, 1353–1357.

Laugerette, F., Vors, C., Géloën, A., Chauvin, M.A., Soulage, C., Lambert-Porcheron, S., Peretti, N., *et al.* (2010) Emulsified lipids increase endotoxemia: possible role in early postprandial low-grade inflammation. *Journal of Nutritional Biochemistry* 22, 53–59.

Leaf, A. and Weber, P.C. (1988) Cardiovascular effects of n-3 fatty acids. *New England Journal of Medicine* 318, 549–557.

Lichtenstein A.H., Brands M., Franch H.A., *et al.* (2006) Diet and lifestyle recommendations revision 2006. A scientific statement from the American Heart Association Nutrition Committee. *Circulation* 114, 82–96.

Lin, R.Y., Choudhury, W., Cai, W., *et al.* (2003) Dietary glycotoxins promote diabetic atherosclerosis in apolipoprotein E-deficient mice. *Atherosclerosis* 168, 213–220.

Liu, J., Zhao, S., Tang, J., *et al.* (2009) Advanced glycation end products and lipopolysaccharide synergistically stimulate proinflammatory cytokine/chemokine production in endothelial cells via activation of both mitogen-activated protein kinases and nuclear factor-kappaB. *FEBS Journal* 276, 4598–4606.

Logsdon, C.D., Fuentes, M.K., Huang, E.H. and Arumugam, T. (2007) RAGE and RAGE ligands in cancer. *Current Molecular Medicine* 7, 777–789.

Ma, L. and Nicholson, L.F. (2004) Expression of the receptor for advanced glycation end products in Huntington's disease caudate nucleus. *Brain Research* 1018, 10–17.

Maillard, L.C. (1912) Action des acids amine sur des sucres: formation des melanoides per voie methodique. *Comptes Rendus de l'Académie des Sciences* 154, 66–68.

Malekinejad, H., Scherpenisse, P. and Bergwerff, A.A. (2006) Naturally occurring estrogens in processed milk and in raw milk (from gestated cows). *Journal of Agricultural and Food Chemistry* 54, 9785–9791.

Matsumoto, T., Tsurumoto, T., Baba, H., *et al.* (2007) Measurement of advanced glycation end products in skin of patients with rheumatoid arthritis, osteoarthritis, and dialysis-related spondyloarthropathy using non-invasive methods. *Rheumatology International* 28, 157–160.

Mattson, M.P. (2003) Will caloric restriction and folate protect against AD and PD? *Neurology* 60, 690–695.

McCarty, M.F. (2005) Secondary hyperparathyroidism promotes the acute phase response – a rationale for supplementing vitamin D in prevention of vascular events in elderly. *Medical Hypotheses* 64, 1022–1026.

McCarty, M.F., Barroso-Aranda, J. and Contreras, F. (2009) The low-methionine content of vegan diets may make methionine restriction feasible as a life extension strategy. *Medical Hypotheses* 72, 125–128.
Meerwaldt, R., Links, T., Graaff, R., *et al.* (2005a) Simple noninvasive measurement of skin autofluorescence. *Annals of the Academy of Sciences* 1043, 290–298.
Meerwaldt, R., Hartog, J.W., Graaff, R., *et al.* (2005b) Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. *Journal of the American Society of Nephrology* 16, 3687–3693.
Meerwaldt, R., Links, T., Zeebregts, C., *et al.* (2008) The clinical relevance of assessing advanced glycation endproducts accumulation in diabetes. *Cardiovascular Diabetology* 7, 29.
Melo, A., Viegas, O., Petisca, C., *et al.* (2008) Effect of beer/red wine marinades on the formation of heterocyclic aromatic amines in pan-fried beef. *Journal of Agricultural and Food Chemistry* 56, 10625–10632.
Meyer, T.E., Kovacs, S.J., Ehsani, A.A., *et al.* (2006) Long-term caloric restriction ameliorates the decline in diastolic function in humans. *Journal of the American College of Cardiology* 47, 398–402.
Miyata, T., Kawai, R., Taketomi, S. and Sprague, S.M. (1996) Possible involvement of advanced glycation end-products in bone resorption. *Nephrology Dialysis Transplantation* 11, 54–57.
Mohr, S.B., Gorham, E.D., Garland, C.F., *et al.* (2006) Are low ultraviolet B and high animal protein intake associated with risk of renal cancer? *International Journal of Cancer* 119, 2705–2709.
Mohr, S.B., Garland, C.F., Gorham, E.D., *et al.* (2007) Is ultraviolet B irradiance inversely associated with incidence rates of endometrial cancer: an ecological study of 107 countries. *Preventive Medicine* 45, 327–331.
Mohr, S.B., Garland, C.F., Gorham, E.D. and Garland, F.C. (2008) The association between ultraviolet B irradiance, vitamin D status and incidence rates of type 1 diabetes in 51 regions worldwide. *Diabetologia* 51, 1391–1398.
Monnier, V.M., Bautista, O., Kenny, D., *et al.* (1999) Skin collagen glycation, glycoxidation, and crosslinking are lower in subjects with long-term intensive versus conventional therapy of type 1 diabetes: relevance of glycated collagen products versus HbA1c as markers of diabetic complications. DCCT Skin Collagen Ancillary Study Group. Diabetes control and complications trial. *Diabetes* 48, 870–880.
Monnier, V.M., Sell, D.R. and Genuth, S. (2005) Glycation products as markers and predictors of the progression of diabetic complications. *Annals of the New York Academy of Sciences* 1043, 567–581.
Moreira, P.I., Smith, M.A., Zhu, X., *et al.* (2005) Oxidative stress and neurodegeneration. *Annals of the New York Academy of Sciences* 1043, 543–552.
Moss, M. and Freed, D.L. (1999) Survival trends, coronary event rates, and the MONICA project. Monitoring trends and determinants in cardiovascular disease. *Lancet* 354, 862–865.
Mulder, D.J., Bieze, M., Graaff, R., *et al.* (2010) Skin autofluorescence is elevated in neovascular age-related macular degeneration. *British Journal of Ophthalmology* 94, 622–625.
Müller, M. and Lier, D. (1994) Fermentation of fructans by epiphytic lactic acid bacteria. *Journal of Applied Bacteriology* 76, 406–411.
Naaber, P., Smidt, I., Stsepetova, J., *et al.* (2004) Inhibition of *Clostridium difficile* strains by intestinal *Lactobacillus* species. *Journal of Medical Microbiology* 53, 551–554.
Nagaraj, R.H., Padmanabha, S., Mailanko, M., *et al.* (2010) Modulation of advanced glycation endproduct synthesis by kynurenines in human lens proteins. *Biochimica et Biophysica Acta* 1804, 829–838.
Nandhini, A.T.A., Thirunavakkarasu, V. and Anuradha, C.V. (2004) Stimulation of glucose utilization and inhibition of protein glycation and AGE products by taurine. *Acta Physiologica Scandinavica* 181, 297–303.
Nandhini, A.T.A., Thirunavakkarasu, V. and Anuradha, C.V. (2005) Taurine prevents collagen abnormalities in high fructose-fed rats. *Indian Journal of Medical Research* 122, 171–177.
Ni, J., Yuan, X., Gu, J., *et al.* (2009) Plasma protein pentosidine and carboxymethyllysine, biomarkers for age-related macular degeneration. *Molecular & Cellular Proteomics* 8, 1921–1933.
Nicolls, M.R. (2004) The clinical and biological relationship between type II diabetes mellitus and Alzheimer's disease. *Current Alzheimer Research* 1, 47–54.
Odetti, P., Angelini, G., Dapino, D., *et al.* (1998) Early glycoxidation damage in brains from Down's syndrome. *Biochemical and Biophysical Research Communications* 243, 849–851.
Odetti, P., Rossi, S., Monacelli, F., *et al.* (2005) Advanced glycation end products and bone loss during aging. *Annals of the New York Academy of Sciences* 1043, 710–717.
Orasanu, G. and Plutzky, J. (2009) The pathologic continuum of diabetic vascular disease. *Journal of the American College of Cardiology* 53, S35–42.
Osawa, T. and Kato, Y. (2005) Protective role of antioxidative food factors in oxidative stress caused by hyperglycemia. *Annals of the New York Academy of Sciences* 1043, 440–451.

Ostergaard, J., Hansen, T.K., Thiel, S. and Flyvbjerg, A. (2005) Complement activation and diabetic vascular complications. *Clinica Chimica Acta* 361, 10–19.

Outwater, J.L., Nicholson, A. and Barnard, N. (1997) Dairy products and breast cancer: the IGF-I, estrogen, and bGH hypothesis. *Medical Hypotheses* 48, 453–461.

Papachroni, K.K., Piper, C., Levidou, G., Korkolopoulou, P., Pawelczyk, L., Diamanti-Kandarakis, E. and Papavassiliou, A.G. (2009) Lysyl oxidase interacts with AGEs signaling to modulate collagen synthesis in polycystic ovarian tissue. *Journal of Cellular and Molecular Medicine* 14, 2460–2469; doi: 10.1111/j.1582-4934.2009.00841.x.

Peppa, M., Brem, P., Ehrlich, J., *et al.* (2003) Adverse effects of glycotoxins on wound healing in genetically diabetic mice. *Diabetes* 52, 2805–2813.

Peskind, E.R., Elrod, R., Dobie, D.J., *et al.* (1998) Cerebrospinal fluid epinephrine in Alzheimer's disease and normal aging. *Neuropsychopharmacology* 19, 465–471.

Platz, E.A., Willett, W.C., Colditz, G.A., *et al.* (2000) Proportion of colon cancer risk that might be preventable in a cohort of middle-aged US men. *Cancer Causes Control* 11, 579–588.

Rahman, I., Biswas, S.K. and Kode, A. (2006a) Oxidant and antioxidant balance in the airways and airway diseases. *European Journal of Pharmacology* 533, 222–239.

Rahman, I., Biswas, S.K. and Kirkham, P.A. (2006b) Regulation of inflammation and redox signaling by dietary polyphenols. *Biochemical Pharmacology* 72, 1439–1452.

Ramamurthy, B., Hook, P., Jones, A.D. and Larsson, L. (2001) Changes in myosin structure and function in response to glycation. *FASEB Journal* 15, 2415–2422.

Rautava, S. and Isolauri, E. (2004) Cow's milk allergy in infants with atopic eczema is associated with aberrant production of interleukin-4 during oral cow's milk challenge. *Journal of Pediatric Gastroenterology and Nutrition* 39, 529–535.

Reddy, G.K. (2004) Cross-linking in collagen by nonenzymatic glycation increases the matrix stiffness in rabbit achilles tendon. *Experimental Diabesity Research* 5, 143–153.

Reeves, M.J. and Rafferty, A.P. (2005) Healthy lifestyle characteristics among adults in the United States 2000. *Archives of Internal Medicine* 165, 854–857.

Rehbein, G., Simm, A. and Hofmann, H.S. (2008) Molecular regulation of S100P in human lung adenocarcinomas. *International Journal of Molecular Sciences* 22, 69–77.

Rosca, M.G., Mustata, T.G., Kinter, M.T., *et al.* (2005) Glycation of mitochondrial proteins from diabetic rat kidney is associated with excess superoxide formation. *American Journal of Physiology - Renal Physiology* 289, F420–F430.

Rüster, M., Franke, S., Späth, M., *et al.* (2005) Detection of elevated N epsilon-carboxymethyllysine levels in muscular tissue and in serum of patients with fibromyalgia. *Scandinavian Journal of Rheumatology* 34, 460–463.

Sancho, A.I., Rigby, N.M., Zuidmeer, L., *et al.* (2005) The effect of thermal processing on the IgE reactivity of the non-specific lipid transfer protein from apple, *Mal d 3. Allergy* 60, 1262–1268.

Sasaki, N., Takeuschi, M., Choei, H., *et al.* (2002) Advanced glycation end products (AGE) and their receptor (RAGE) in the brain of patients with Creutzfeldt-Jacob disease with prion plaques. *Neuroscience Letters* 326, 117–120.

Sbarbati, A., Valleta, E., Bertini, M., *et al.* (2003) Gluten sensitivity and 'normal' histology: is the intestinal mucosa really normal? *Digestive and Liver Disease* 35, 768–773.

Schepers, E., Glorieux, G. and Vanholder, R. (2010) The gut: the forgotten organ in uremia? *Blood Purification* 29, 130–136.

Schmidt, A.M., Vianna, M., Gerlach, M., *et al.* (1992) Isolation and characterization of binding proteins for advanced glycosylation endproducts from lung tissue which are present on the endothelial cell surface. *Journal of Biological Chemistry* 267, 14987–14997.

Schmidt, A.M., Yan, S.D., Brett, J., *et al.* (1993) Regulation of mononuclear phagocyte migration by cell surface binding proteins for advanced glycosylation endproducts. *Journal of Clinical Investigation* 92, 2155–2168.

Schmidt, A.M., Mora, R., Cao, R., *et al.* (1994a) The endothelial cell binding site for advanced glycation end products consists of a complex: an integral membrane protein and a lactoferrin-like polypeptide. *Journal of Biological Chemistry* 269, 9882–9888.

Schmidt, A.M., Hasu, M., Popov, D., *et al.* (1994b) The receptor for Advanced Glycation End products (AGEs) has a central role in vessel wall interactions and gene activation in response to AGEs in the intravascular space. *Proceedings of the National Academy of Sciences USA* 91, 8807–8811.

Schmidt, A.M., Yan, S.D., Yan, S.F. and Stem, D.M. (2001) The multiligand receptor RAGE is a progression factor amplifying immune and inflammatory responses. *Journal of Clinical Investigation* 108, 949–955.

Sebekova, K., Krajcoviova-Kudlackova, M., Schinzel, R., *et al.* (2001) Plasma levels of advanced glycation end products in healthy, long-term vegetarians and subjects on a western mixed diet. *European Journal of Nutrition* 40, 275–281.

Sebekova, K., Kupcova, V., Schinzel, R. and Heidland, A. (2002) Markedly elevated levels of plasma advanced glycation end products in patients with liver cirrhosis – amelioration by liver transplantation. *Journal of Hepatology* 36, 66–71.

Semba R.D., Bandinelli, S., Sun, K., *et al.* (2009) Plasma carboxymethyl-lysine, an advanced glycation end product, and all-cause and cardiovascular disease mortality in older community-dwelling adults. *Journal of the American Geriatric Society* 57, 1874–1680.

Smith, M.A., Taneda, S., Rickey, P.L., *et al.* (1994) Advanced Maillard reaction end products are associated with Alzheimer pathology. *Proceedings of the National Academy of Sciences USA* 91, 5710–5714.

Soldatos, G., Cooper, M.E. and Jandeleit-Dahm, K.A. (2005) Advanced-glycation end products in insulin-resistant states. *Current Hypertension Reports* 7, 96–102.

Stamatas, G.N., Estanislao, R.B., Suero, M., *et al.* (2006) Facial skin fluorescence as a marker of the skin's response to chronic environmental insults and its dependence on age. *British Journal of Dermatology* 154, 125–132.

Stampfer, M.J., Hu, F.B., Manson, J.E., *et al.* (2000) Primary prevention of coronary heart disease in women through diet and lifestyle. *New England Journal of Medicine* 343, 16–22.

Steenvoorden, M.M., Huizinga, T.W., Verzijl, N., *et al.* (2006) Activation of receptor for advanced glycation end products in osteoarthritis leads to increased stimulation of chondrocytes and synoviocytes. *Arthritis & Rheumatism* 54, 253–263.

Stitt, A.L. (2005) The Maillard reaction in eye disease. *Annals of the New York Academy of Sciences* 1043, 582–597.

Sun A.Y., Wang, Q., Simonyi, A. and Sun, G.Y. (2010) Resveratrol as a therapeutic agent for neurodegenerative diseases. *Molecular Neurobiology* 41, 375–83.

Sunahori, K., Yamamura, M., Yamana, J., *et al.* (2006) Increased expression of receptor for advanced glycation end products by synovial tissue macrophages in rheumatoid arthritis. *Arthritis & Rheumatism* 54, 97–104.

Suzuma, K., Otani, A., Oh, H., *et al.* (1999) 17- Beta-estradiol increases VEGF receptor-2 and promotes DNA synthesis in retinal microvascular endothelial cells. *Investigative Ophthalmology & Visual Science* 40, 2122–2129.

Takada, M., Hirata, K., Ajiki, T., Suzuki, Y. and Kuroda, Y. (2004) Expression of receptor for advanced glycation end products (RAGE) and MMP-9 in human pancreatic cancer cells. *Hepatogastroenterology* 51, 928–930.

Taki, K., Takayama, F., Tsuruta, Y. and Niwa, T. (2006) Oxidative stress, advanced glycation end product, and coronary artery calcification in hemodialysis patients. *Kidney International* 70, 218–224.

Tateno, T., Ueno, S. and Hiwatashi, K. (2008) Expression of receptor for advanced glycation end products (RAGE) is related to prognosis in patients with esophageal squamous cell carcinoma. *Annals of Surgical Oncology* 16, 440–446.

Tavan, E., Cayuela, C., Antoine, J.M. and Cassand. P. (2002) Antimutagenic activities of various lactic acid bacteria against food mutagens: heterocyclic amines. *Journal of Dairy Research* 69, 335–341.

Tezel, G., Luo, C. and Yang, X. (2007) Accelerated aging in glaucoma: immunohistochemical assessment of advanced glycation end products in the human retina and optic nerve head. *Investigative Ophthalmology & Visual Science* 48, 1201–1211.

Thiel, R. and Fowkes, S.W. (2005) Can cognitive deterioration associated with Down syndrome be reduced? *Medical Hypotheses* 64, 524–532.

Tikellis, C., Cooper, M.E. and Thomas, M.C. (2006) Role of the renin-angiotensin system in the endocrine pancreas: implications for the development of diabetes. *The International Journal Of Biochemistry & Cell Biology* 38, 737–751

Tikellis, C., Thomas, M.C., Harcourt, B.E., Coughlan, M.T., Pete, J., Bialkowski, K., Tan, A., *et al.* (2008) Cardiac inflammation associated with a Western diet is mediated via activation of RAGE by AGEs. *American Journal of Physiology - Endocrinology and Metabolism* 295, E323–330.

Tlaskalová-Hogenová, H., Tucková, L., Stepánková, R., *et al* (2005) Involvement of innate immunity in the development of inflammatory and autoimmune diseases. *Annals of New York Academy of Science* 1051, 787–798.

Tokita, Y., Hirayama, Y., Sekikawa, A., *et al.* (2005) Fructose ingestion enhances atherosclerosis and deposition of advanced glycated end-products in cholesterol-fed rabbits. *Journal of Atherosclerosis and Thrombosis* 12, 260–267.

Tsukahara, H., Shibata, R., Ohta N., *et al.* (2003) High levels of urinary pentosidine, an advanced glycation end product, in children with acute exacerbation of atopic dermatitis: relationship with oxidative stress. *Metabolism* 52, 1601–1605.

Tucker, K.L., Morita, K., Qiao, N., *et al.* (2006) Colas, but not other carbonated beverages, are associated with low bone mineral density in older women: The Framingham Osteoporosis Study. *American Journal of Clinical Nutrition* 84, 936–942.

Unno, Y., Sakai, M., Sakamoto, Y., *et al.* (2004) Advanced glycation end products-modified proteins and oxidized LDL mediate down-regulation of leptin in mouse adipocytes via CD36. *Biochemical and Biophysical Research Communications* 325, 151–156.

Uribarri, J. and Tuttle, K.R. (2006) Advanced glycation end products and nephrotoxicity of high-protein diets. *Clinical Journal of the American Society of Nephrology* 1, 1293–1299.

Uribarri, J., Woodruff, S., Goodman, S., *et al.* (2010). Advanced glycation end products in foods and a practical guide to their reduction in the diet. *Journal of American Dietetic Association* 110, 911–916.

US Department of Health and Human Services, National Institutes of Health, and National Heart, Lung, and Blood Institute (2006) *Your Guide to Lowering Your Blood Pressure with Dietary Approach to Stop Hypertension (DASH).* NIH Publication 06-4082, revision of 1st ed. (1988).

Visentin, S., Medana, C., Barge, A., *et al.* (2010) Microwave-assisted Maillard reactions for the preparation of advanced glycation end products (AGEs). *Organic & Biomolecular Chemistry* 21, 2473–2477.

Vitek, M.P., Bhattacharya, K., Gendening, J.M., *et al.* (1994) Advanced glycation end products contribute to amyloidosis in Alzheimer disease. *Proceedings of the National Academy of Sciences USA* 91, 4766–4770.

Vlassara, H. (2005) Advanced glycation in health and disease. Role of the modern environment. *Annals of the New York Academy of Sciences* 1043, 452–460.

Vlassara, H., Cai, J., Crandall, J., *et al.* (2002) Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. *Proceedings of the National Academy of Sciences USA* 99, 15596–15601.

Vlassara, H., Torreggiani, M., Post, J.B., *et al.* (2009) Role of oxidants/inflammation in declining renal function in chronic kidney disease and normal aging. *Kidney International Supplement* 14, S3–11.

Wiame, E., Delpierre, G., Collard, F. and Van Schaftingen, E. (2002) Identification of a pathway for the utilization of the Amadori product fructoselysine in Escherichia coli. *Journal of Biological Chemistry* 277, 42523–42529.

Yagmur, E., Tacke., F., Weiss, C.P., *et al.* (2006) Elevation of Nepsilon-(carboxymethyl)lysine-modified advanced glycation end products in chronic liver disease is an indicator of liver cirrhosis. *Clinical Biochemistry* 39, 39–45.

Yamagishi, S., Fujimori, H., Yonekura, H., *et al.* (1998) Advanced glycation end products inhibit prostacyclin production and induce plasminogen activator inhibitor-1 in human microvascular endothelial cells. *Diabetologica* 41, 1435–1441.

Yan, S.F., Ramasamy, R. and Schmidt, A.M. (2009) Receptor for AGE (RAGE) and its ligands-cast into leading roles in diabetes and the inflammatory response. *Journal of Molecular Medicine* 87, 235–247.

Zeng, S., Feirt, N., Goldstein, M., *et al.* (2004) Blockade of receptor for advanced glycation end product (RAGE) attenuates ischemia and reperfusion injury to the liver in mice. *Hepatology* 39, 422–432.

Zheng, F., He, C., Cai, W., *et al.* (2002) Prevention of nephropathy in mice by a diet low in glycoxidation products. *Diabetes/Metabolism Research and Reviews* 18, 224–237.

Zimmerman, G.A., Meistrell, M., Bloom, O., *et al.* (1995) Neurotoxicity of advanced glycation endproducts during focal stroke and neuroprotective effects of aminoguanidine. *Proceedings of the National Academy of Sciences USA* 92, 3744–3748.

Zittermann, A., Schleithoff, S.S. and Koerfer, R. (2005) Putting cardiovascular disease and vitamin D insufficiency into perspective. *British Journal of Nutrition* 94, 483–492.