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Review

Dietary ALEs are a risk to human health – NOT!

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Advanced lipoxidation end-products (ALEs) are formed by reaction of protein with lipid-derived reactive peroxyl and carbonyl compounds produced during food processing and cooking. There is concern that ALEs may induce damage in the gastrointestinal tract, affecting gut health, or enter the body and promote vascular inflammation and tissue damage. However, there is no direct evidence that ALE-proteins are a source of damage in the intestines or that they are transported into the circulation and cause pathology. Modification of proteins by ALEs impedes their digestion, and reactive ALEs released by gastrointestinal proteases would react with proteins or peptides in the gut, limiting their absorption. There are also potent enzymatic mechanisms for detoxifying ALEs or their precursors prior to their entry into the circulation. If ALEs gain access to the circulation, a battery of protective enzymes in tissue provides a second level of defense. These enzymes may be induced in intestinal epithelia and liver by low doses of ALEs, and adaptive responses would provide enhanced protection against future exposure to ALEs. Overall, except in persons with compromised organ function, *e.g.*, vascular, hepatic, or renal diseases, there is little evidence that food ALEs will have any significant pathological effects.

Keywords: Advanced lipoxidation end product / Food absorption / Food detoxification / Food digestion / Inflammation

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This article focuses on contra arguments about "Dietary ALEs are a risk to human health".
Introduction: http://dx.doi.org/10.1002/mnfr.2007000030
Pro arguments: http://dx.doi.org/10.1002/mnfr.200600303

1 Introduction

The purpose of this debate is to discuss the evidence that dietary advanced lipoxidation end-products (ALEs) are a risk to human health. In other words, is there evidence that chronic exposure to dietary ALEs leads to disease, to include intestinal, hepatic, renal, vascular, and neurodegenerative diseases, or even milder pathologies such as insulin resistance and metabolic syndrome? At the outset, it is helpful to define a few terms. First, lipid peroxidation products (LPOs) are the immediate products of oxidation of lipids by a variety of reactive oxygen species (ROS) (Fig. 1). LPOs include lipid peroxides and epoxides and their degradation products, including saturated and unsaturated aldehydes and ketones. The formation, rearrangement, and degradation of LPOs may be catalyzed by free metal ions, or metalloproteins and lipid-soluble metal complexes such as hemoglobin and heme in food products.

LPOs are the precursors of ALEs, which are formed by reaction of LPOs with protein (Fig. 1). ALEs may be labile or stable adducts or crosslinks in protein, some of which may be colored or fluorescent. Some ALEs, such as N^{ε}-(carboxymethyl)lysine (CML) and N^{ε}-(carboxyethyl)lysine (CEL) may be chemically inert, while others, such as the malondialdehyde (MDA) adduct to lysine and 4-hydroxynonenal (HNE) adducts to cysteine, histidine, and lysine may have reactive functional groups. It is possible that both the inert and reactive ALEs may be pathologically significant.

With regard to this debate, the focus is on the toxicity of ALEs, not LPOs. Thus, despite evidence that some LPOs, such as lipid peroxides, may be absorbed and transported on plasma lipoproteins [1, 2], and may induce inflammatory

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Abbreviations: AGE, advanced glycation end-product; **ALE,** advanced lipoxidation end-product; **CML,** N^{ε}-(carboxymethyl)lysine; **GPx,** glutathione peroxidases; **GSH,** glutathione

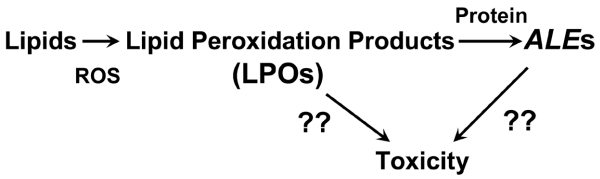

Figure 1. Relationship between LPOs and ALEs. LPOs are the early products of lipid peroxidation. ALEs are products of reaction between LPOs and protein. Both LPOs and ALEs may contribute to an increase in toxicity of food products during cooking, processing, or storage.

responses [3, 4], the following discussion will address the toxicity of ALEs, not LPOs. This, unfortunately, is a logical, rather than a practical distinction, since LPOs and ALEs will always be present together in foods, making it difficult to distinguish between the effects of LPOs *versus* ALEs. Conclusions regarding the toxicity of ALEs are also compromised by the fact that cooked foods rarely contain only LPOs or ALEs. Carbohydrates in foods also react to form advanced glycation end-products (AGEs), in concert with the formation of ALEs. Thus, it is nearly impossible to do a "clean experiment" in which foods are enriched with pure ALEs, but not LPOs or AGEs. In the normal diet, these compounds are also accompanied by oxidized nucleic acids, coenzymes, and vitamins, which may have their own toxicity profile. All of these "contaminants" complicate the interpretation of experiments regarding the toxicity of ALEs. Despite the experimental challenges, however, there are several lines of evidence which suggest that ALEs are unlikely to be, or are, nontoxic.

2 What makes a compound toxic?

Before addressing the toxicity of ALEs, it would be useful to frame this discussion with a few guidelines (Table 1). First, everything is toxic at a sufficiently high concentration, even essentials of life such as oxygen, water, and salt. Thus, in discussion of the toxicity of ALEs, the focus should be on levels of ALEs likely to be present in foods. Foods with higher levels of ALEs might, in fact, have sensory properties, such as an unacceptable appearance, consistency, or flavor, which would discourage their consumption. A second consideration is that even potentially toxic compounds such as H_2O_2 and HOCl, may be controlled or domesticated under physiological conditions. Indeed, H_2O_2 is an intermediate in signal transduction during stress [5],

Table 1. Some general principles regarding toxicity

Everything is toxic at high concentration
Nothing is toxic unless it actually causes damage
There are endogenous defenses against toxins
Low levels of toxins may actually be protective

while HOCl has a role in protection against microbial infection and may also be involved in the oxidation and turnover of cellular debris [6]. Thus, the fact that a compound may be reactive does not necessarily imply that it is toxic or causes damage under biological conditions. This principle is particularly relevant to the detection of lipid peroxides in plasma – the fact that these compounds are present is an indication that they have not reacted with biomolecules, *i. e.*, that they may be relatively inert *in vivo*, awaiting inactivation by physiological defense systems. This leads to a third consideration, that there are powerful endogenous defenses against common toxins. These include enzymes such as catalase, superoxide dismutase, glutathione (GSH)-dependent peroxidases (GPx), reductases and *S*-transferases (GST), aldehyde reductases and dehydrogenases, the glyoxalase pathway, and cytochrome P450 oxidoreductases, all of which participate in the detoxification of dietary components. These enzymes are present in all nucleated cells in the body; those in intestinal epithelia assist in detoxification of LPOs and their degradation products during transport into plasma and lymph [7–9], while enzymes in other tissues, particularly the liver, provide a second level of protection. Lipid peroxides that escape reduction in the intestines may be reduced and inactivated by extracellular GPx in plasma. Plasma proteins, such as albumin, also play an important passive role in detoxification by reacting with and trapping reactive peroxyl and carbonyl compounds, preventing their uptake into tissues. In *in vitro* experiments demonstrating the toxicity of extracellular carbonyl compounds, it is not uncommon to use serum-free medium [10] – serum proteins react with reactive carbonyl compounds, inhibiting their cytotoxicity. Peroxyl radicals derived from lipid hydroperoxides are also reduced by methionine residues in HDL [11], yielding methionine sulfoxide and hydroxylipids such as 9- and 13-hydroxyoctadecadienoic acids from linolenic acid. These hydroxylipids and the oxidation product, methionine sulfoxide, are present in plasma and HDL, respectively, and are likely to increase with exposure to diets rich in LPOs and ALEs. Finally, it is worth noting that low levels of a toxin are tolerable, *i. e.*, there are threshold effects. Indeed, low-level exposure may be protective by inducing defensive responses. Low levels of heavy metals induce the synthesis of GSH in endothelial cells, providing enhanced protection against future exposure to concentrations of toxic metal ions [12] that would otherwise be lethal to cells. Electrophiles, LPOs and probably ALEs also induce protection through oxidative stress and the electrophile-response element, inducing enzymes

that protect against both acute and chronic exposure to dietary components [13]. These endogenous protective responses raise toxicity thresholds, limiting potential damage from dietary components. GSH-dependent enzymes appear to be especially important since disruption of intestinal GPx leads to colitis and cancer [14, 15]. Overall, as summarized in Table 1, there are a number of protective mechanisms that limit the potential toxicity of dietary ALEs. However, assuming that they may be toxic, the next section considers a number of factors that would limit exposure to these compounds.

3 Protections against the toxicity of ALEs

In the absence of a body of direct evidence on the toxicity of dietary ALEs, some of the following discussion is admittedly, but necessarily, speculative, and based on the author's personal opinions and assumptions. However, the discussion is designed to identify a number of specific issues that must be addressed before the toxicity of ALEs can be accepted. The toxicity of ALEs depends on a number of events: their digestion, absorption, detoxification, and reactivity (Table 2). Digestibility is an important consideration. The more cooked and modified proteins are, the more slowly they are degraded in the gastrointestinal tract. Many LPOs, for example, react with lysine and arginine residues in food proteins, limiting their digestion by trypsin. Aggregation of cooked and ALE-modified protein may also interfere with their solubilization for enzymatic digestion. The outcome is that highly modified, ALE-rich proteins are not efficiently digested in the gastrointestinal tract. Their indigestibility limits their absorption, and, in effect, they become roughage. Even in those cases in which ALEs are released from proteins, it is unlikely that they would be absorbed. In some cases, they would be trapped by reaction with proteins, peptides, or amino acids in the gastrointestinal tract.

In those cases in which low molecular weight ALEs are generated by proteolytic digestion, only a small fraction is likely to be absorbed. Nonpolar compounds may be absorbed by pathways similar to those involved in drug delivery, but most ALEs are likely to be polar compounds, formed from amino acids and oxidized lipids. Little is known about the uptake of modified, polar amino acids, but the limited absorption of these compounds by intestinal transporters would decrease their potential toxicity. ALEs that are absorbed may, of course, be detoxified by conversions in either the intestinal epithelia or in cells of various organs, including endothelial and parenchymal cells. However, it is likely that gut epithelia would be exposed to the highest concentrations of toxic ALEs and that these cells would absorb the brunt of the toxicity. These cells have among the highest turnover rates in the body and constantly slough off into the gastrointestinal tract, providing a convenient protective absorbent for toxic molecules. Indeed, irritation of the intestinal wall might enhance the elimination of toxic ALEs. Those ALEs which make it through these various screens and are absorbed into blood or lymph would then be subject to hepatic detoxification and renal elimination, further limiting potential toxicity to tissues. Despite these defenses, it is likely that some dietary ALEs, in the form of amino acid adducts, will be absorbed – there are many studies documenting the appearance of dietary AGEs in the circulation following an AGE-rich meal. However, there is no direct evidence on the toxicity of low molecular weight AGEs or ALEs, *e.g.*, CML or CEL. Studies with MDA-lysine and HNE-adducts to amino acids could address this issue, but these reactive compounds would have to be administered as part of a complete diet.

Table 2. Factors limiting toxicity of ALEs

Limited digestion of ALE-proteins by gastrointestinal protease
Trapping of reactive ALEs by proteins in the gastrointestinal tract
Detoxification of ALEs by enzymes in intestinal epithelia
Poor absorption of intact ALE-proteins or degradation products
Detoxification of ALEs by in liver and other tissues

One other factor that should be considered in evaluating the toxicity of ALEs is their interaction with cellular receptors. CML, which is both an AGE and an ALE [16], is absorbed from the diet and could react with AGE or scavenger receptors, such as the RAGE (receptor for AGE), and induce proinflammatory signal transduction cascades [17]. While ligation to receptors may contribute to the toxicity of chemically inert ALEs, scavenger receptors generally require multivalent ligands. Another presentation in this series [18] points out the requirement for aggregation of AGEs in order to ligate RAGE. However, it is unlikely that intact, multivalent ALE-proteins that survive digestion in the gastrointestinal tract would then be absorbed and enter the circulation in the form of an aggregate or polyvalent ligand. It seems equally unlikely that dietary ALEs would induce a significant proinflammatory response in the vasculature or tissues. Overall, as summarized in Table 2, even if one accepts the hypothesis that ALEs are toxic, or acknowledges their toxicity in cellular systems *in vitro*, it is unlikely that they would be absorbed into the circulation and induce toxic and inflammatory responses.

4 Interpretation of inflammatory responses to diet

There is some evidence that diets rich in AGEs and ALEs induce inflammatory responses [19–21], as measured by increases in inflammatory biomarkers in plasma and progression of vascular disease in either human subjects or animal models. In some cases, concerns about experimental

design undermine the relevance of these studies, for example: the development of vascular disease in response to intravenous injection of AGEs; the development of vascular disease in genetically compromised animal models; or an increase in inflammatory cytokines in response to administration of heated (oxidized) oils by gavage. Conclusions based on these types of experiments are, from a dietary perspective, difficult to swallow. In other cases, the toxicity of AGEs and/or ALEs, measured by an increase in inflammatory biomarkers in plasma, is reported in diabetic patients with renal and/or vascular complications [22 – 24]. The relevance of these studies to risk in a healthy population is uncertain. Fatty meals, even in healthy subjects, cause an increase in circulating lipid peroxides [25], a transient impairment of endothelial function [26], and also a significant increase in circulating levels of several inflammatory mediators, including IL-6, IL-18, TNF-α, ICAM-1, and VCAM-1 [27]. Interestingly, many of these changes in inflammatory mediators are preventable by supplementation with antioxidant vitamins (C and E) [27, 28], suggesting that a balanced, nutritious diet may suppress any LPO- or ALE-dependent inflammatory processes. A nutritious diet is an essential part of the experimental design for testing the toxicity of ALEs (see Section 5)! It is not clear, however, whether suppression of the inflammatory response is good or bad. In some of the cases, for example following exercise, the increase in IL-6, which has been described as a "carbohydrate sensor" [29], induces a shift toward lipolysis in adipose tissue and lipid metabolism in muscle [30]. A high level of IL-6 in response to an ALE-rich diet might also promote lipid disposal. Other so-called inflammatory mediators may have regulatory roles in metabolic adaptation following lipid- or ALE-rich diets, independent of inflammation and tissue injury or toxicity. Until specific ALEs are isolated and tested, it will be difficult to draw firm conclusions from these studies.

5 Alternative hypotheses

Highly cooked, fatty foods are not a component of the food pyramid and should not be a foundation for a healthy diet. However, their unhealthiness may not be the result of their ALE content. These foods are, by nature, rich in fats and calories, whose independent impact on health and obesity cannot be ignored. In addition, highly cooked, fatty foods would have lower nutritional value because of increased chemical modification of essential amino acids – lysine, arginine, histidine, and tryptophan – by LPOs [31]. The bioavailability of these and other essential amino acids may also be limited because of decreased digestibility of the modified proteins. In addition to effects on amino acid availability, cooking of foods also decreases their content of essential fatty acids and several vitamins. Fatty foods tend to be rich in relatively inert, saturated fats – they contain only low levels of linolenic and linolenic acid and these are readily oxidized during cooking. The destruction of these essential fatty acids has potential downstream effects on prostaglandin and leukotriene metabolism, affecting vascular and neuronal health and inflammatory responses. Some vitamins, such as ascorbic acid, are decomposed by heating, while pyridoxine, thiamine, riboflavin, and other nutrients, such as pantothenic acid and carnitine, contain reactive functional groups and may be modified by Maillard reactions during cooking. Lipophilic antioxidants, such as carotene and tocopherols, may also be consumed during prooxidant reactions involved in formation of ALEs. The uptake of all of the lipophilic vitamins may also be impaired by poor fat digestion and steatorrhea following a fat-rich meal. Thus, there are a number of factors that may contribute to the toxicity of high-ALE diets, independent of their ALE content, and long-term pathology may be more the result of the poor nutritional value of the food, rather than its ALE content. This is an important point, often overlooked in the interpretation of experiments on the toxicity of ALE-rich diets, whether in a healthy or diseased population.

6 Summary

Cooking food, like most human activities, has a risk/benefit ratio. Cooking destroys pathogens, aids in food preservation, and improves flavor, aroma, and texture. At the same time, it partially destroys some labile components, and reduces digestibility and absorption of food proteins. Cooking also produces ALEs, some of which may be toxic, carcinogenic or, at best, distasteful. However, there is little evidence that ALEs, at concentrations found normally in cooked foods, pose a significant risk to human health in a healthy population. Evidence that dietary ALEs are toxic is frequently based on poor experimental design, using inappropriate amounts or routes of administration of ALEs and foods deficient in essential amino and fatty acids and vitamins. Many studies purporting to demonstrate the toxicity of ALEs are conducted in compromised animals or in human subjects with vascular or renal disease, atherosclerosis or diabetes. In normal, healthy subjects on a diet rich in fresh fruits and vegetables, there is limited evidence to support the argument that ALEs are a risk factor for disease.

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