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Isolevuglandins and Cardiovascular Disease

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Abstract

Isolevuglandins are 4-ketoaldehydes formed by peroxidation of arachidonic acid. Isolevuglandins react rapidly with primary amines including the lysyl residues of proteins to form irreversible covalent modifications. This review highlights evidence for the potential role of isolevuglandin modification in the disease processes, especially atherosclerosis, and some of the tools including small molecule dicarbonyl scavengers utilized to assess their contributions to disease.

Keywords

Lipid peroxidation; reactive lipid species; isolevuglandins; cardiovascular disease; HDL

Introduction

Lipid peroxidation is a constant process within living cells, as a wide variety of cellular processes produce reactive oxygen species (ROS). However, initiating events of pathological processes often exacerbate rates of lipid peroxidation, resulting in accumulation of lipid peroxidation products that contribute to the progression of disease. While abundant in phospholipids of cellular membranes, polyunsaturated fatty acids (PUFAs) are highly susceptible to peroxidation by ROS such as the hydroxyl radical. Peroxidation of linoleic acid and arachidonic acid generates a variety of stable as well as reactive products (Figure 1). This mini-review will focus on one particular family of lipid peroxidation products, the isolevuglandins (IsoLG), and their role in cardiovascular disease.

IsoLGs are a family of 4-ketoaldehydes (alternatively referred to as isoketals) that form from the peroxidation of arachidonic acid through bicycloendoperoxide intermediates (termed H2-isoprostanes) and undergo concerted rearrangement of the bicycloendoperoxide group to form the 4-ketoaldehyde[1, 2]. Because abstraction of the initial hydrogen can occur at 3 different positions on arachidonic acid, subsequent rearrangement and addition of oxygen give rise to four regioisomers of H_2-isoprostanes. Each of these regioisomers can give rise to two distinct 4-ketoaldehyde isomers, generating a total of eight regioisomers (and 64 stereoisomers) of IsoLG (Figure 2). While all regioisomers form to some extent, the formation of certain regioisomers are favored (e.g. 15-E_2-IsoLG and 5-E_2-IsoLG versus 8-E_2-IsoLG and 12-E_2-IsoLG).

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A number of biological enzymes generate IsoLG, with the most significant being myeloperoxidase, NADPH oxidase (NOX), and cyclooxygenase. Myeloperoxidase is an abundant heme protein secreted by phagocytes in response to stimulation that serves as an enzyme catalyst for lipid peroxidation and lipoprotein oxidation *in vivo*[3]. Formation of IsoLG adducts by the myeloperoxidase/H_2O_2 system of leukocytes was found in a Candida sepsis murine model of inflammation[4]. Myeloperoxidase forms IsoLG adducts in lysophosphatidylethanolamines[5] and also in proteins and phosphatidylethanolamines in high density lipoprotein[6]. The primary function of the NOX family is the generation of ROS, which directly leads to IsoLG formation via free radicals[7]. However, NOX activation can also lead to cyclooxygenase activation[8] and thereby produce IsoLG via that pathway. Unlike the other enzymes mentioned, cyclooxygenase can not only generate a lipid peroxide directly (rather than indirectly by reaction between ROS and PUFA) but also generate prostaglandin (PG) H_2. If not rapidly converted to other prostaglandins by PG synthases, PGH_2 will undergo spontaneous non-enzymatic rearrangement to form levuglandin E_2 and levuglandin D_2, which are two specific stereoisomers of 15-E_2-IsoLG and 15-D_2-IsoLG, respectively. Following cyclooxygenase activity, IsoLG forms protein adducts in cells[9] and in tissues[10] as well as bind to histones[11].

Two characteristics distinguish IsoLGs from other common lipid peroxidation species such as 4- hydroxynonenal (HNE), acrolein, and malondialdehyde (MDA). First, while all of these lipid peroxidation products spontaneously react with nucleophiles, IsoLGs are at least an order of magnitude more reactive with proteins than MDA or HNE[12]. Second, IsoLG almost exclusively react with primary amines[12] (e.g. lysine and phosphatidylethanolamine) and not other nucleophiles like thiols (e.g. cysteine) or indoles (e.g. histidine). Both characteristics can be rationalized based on the mechanism of their chemical reaction. Characteristic of aldehydes, the aldehyde group of IsoLGs rapidly react with primary amines (not other nucleophiles like thiols) to form a highly reversible imine adduct (Schiff Base) (Figure 3). The 4-keto moiety of IsoLG subsequently reacts with the nitrogen of the imine, generating a dihydropyrrolidine adduct which then loses two water molecules to form the essentially irreversible pyrrole adduct[13]. Under oxidizing conditions, this pyrrole adduct can either react with molecular oxygen to form lactam and hydroxylactam adducts or with other nucleophiles such as thiols or nearby pyrroles to form intermolecular crosslinks. The latter reaction explains the high propensity of IsoLGs to crosslink proteins which distinguishes them from other reactive lipids species like HNE. Due to their rapid reaction rates, their propensity to form protein crosslinks, and the formation of unique adduct structures, IsoLGs often exert very different effects than other products of lipid peroxidation. The following sections of this review will discuss how IsoLG modification of various proteins including plasma lipoproteins appears to play an important role in the development of diseases related to oxidative stress.

Risk factors for cardiovascular disease elevate IsoLG levels

Any claim that the formation of IsoLG adducts plays a causative role in disease processes requires the following evidence: 1) IsoLG adducts are elevated during the development of disease, 2) preventing the formation of these adducts attenuates disease, and 3) IsoLG exerts detrimental effects relevant to the development of disease. Furthermore, the ability to

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accurately quantify levels of IsoLG-adducts is critical. In this section, we will briefly review current approaches to measure IsoLG-protein adducts and evidence to support that IsoLG adduct formation contributes to cardiovascular diseases.

Two major approaches have been used to quantitate IsoLG-protein adducts: isotope dilution mass spectrometry and antibody-based approaches such as enzyme-linked immunosorbent assay (ELISA), immunohistochemistry, and flow cytometry. Isotope dilution mass spectrometry is a highly specific and quantitative method, where protein precipitated from tissues are digested to constituent IsoLG-modified lysines and measured against an isotopically-labeled internal standard[12, 14]. Similar mass spectrometry approaches are used to measure IsoLG-phosphatidylethanolamine adducts[5]. However, one disadvantage is that this approach cannot identify the specific protein modified by IsoLG nor determine the spatial location of adducts in tissue. These questions could potentially be addressed by mass spectrometry techniques used for detecting post-translational modifications on whole proteins or tryptic digests or by Matrix Assisted Laser Desorption/Ionization (MALDI) imaging techniques. However, such strategies have not been proved feasible for IsoLG adducts in tissues, so further development of these techniques is needed. To address questions of spatial localization of adducts in tissue, investigators have generated antibodies against IsoLG adducts[15, 16]. These antibodies have been particularly useful in immunochemistry to identify cellular sites of adduct formation and in flow cytometry to measure specific cell types where IsoLG adducts are produced. However, one challenge with these approaches is that current antibodies are of relatively low affinity, making approaches such as immunoprecipitation infeasible. For these reasons, the development of additional anti-IsoLG adduct antibodies is critically needed.

The earliest evidence for increased IsoLG adducts in cardiovascular disease came from ELISA studies performed with anti-IsoLG adduct antibodies on plasmas of patients with and without verified atherosclerosis. IsoLG-protein adducts were found to be elevated two-fold in diseased patients[17]. IsoLG adducts are also elevated in conditions that increase risk for cardiovascular disease including sepsis, hypertension, hypercholesterolemia, obesity, chronic kidney disease. Poliakov et al. showed that mice with sepsis induced by *Candida* had a 3-fold elevation in plasma IsoLG-protein adduct levels[4]. As detailed in their article in this issue, Kirabo et al. demonstrated that levels of IsoLG adducts were increased in several murine models of hypertension and elucidated some mechanisms whereby IsoLG contributes to hypertension[18–20]. We found that IsoLG adducts of both protein and phosphatidylethanolamine were elevated in high density lipoprotein (HDL) isolated from humans with familial hypercholesterolemia compared to normal control subjects[21, 22]. The consequences of this modification on the function of HDL is discussed in more detail below. We also found increases in IsoLG-phosphatidylethanolamine adducts in livers of diet-induced obese mice[22]. Salomon and colleagues found increased IsoLG adducts in patients with chronic kidney disease[23]. They also found increased IsoLG adducts in mice chronically exposed to alcohol[5]. Although more studies are needed to fully elucidate the relationship between elevations in IsoLG adducts and development of cardiovascular disease, these studies support the notion that risk factors for cardiovascular disease elevate IsoLG adduct levels and may therefore play a causal role, rather than simply being a tombstone marker of well-established disease.

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The formation of IsoLG adducts is not isolated to cardiovascular disease only. Any tissue with significant arachidonic acid content is prone to oxidative damage and will likely have elevated levels of IsoLG adducts. Other conditions where increased IsoLG adducts have been detected include cancer[24], Alzheimer's disease[25], idiopathic pulmonary fibrosis[7], and retinopathy[26].

Development of dicarbonyl scavengers to assess the role of IsoLG in disease

Determining the contribution of IsoLG to disease processes requires effective interventions that selectively reduce levels of IsoLG adducts. In theory, increasing tissue levels of antioxidants could reduce IsoLG formation. However, IsoLG formation in tissues appears to be driven by ROS producing enzymes (such as myeloperoxidase and NOX), so that simply increasing tissue concentrations of antioxidants is unlikely able to combat the formation of IsoLG that result from these activated enzymes. Even with supraphysiological levels of antioxidants that may block IsoLG formation, this would also block the generation of hydrogen peroxide and a plethora of other lipid peroxidation products. Such broad approach would not only make interpreting the mechanism of IsoLG difficult, it would also drive adverse physiological effects since hydrogen peroxide acts as an intracellular signaling molecule that initiates several protective responses[27]. Such adverse effects may underlie the failure in clinical trials using dietary antioxidants like vitamin C and vitamin E in producing significant improvements in endpoints of cardiovascular disease[28, 29].

To effectively inhibit the formation of IsoLG adducts, we have focused on developing small molecule primary amines that act as IsoLG scavengers[30–32]. These scavengers do not alter the formation of IsoLG or other lipid peroxidation products like F_2-isoprostanes, nor do they block the formation of important signaling molecules like hydrogen peroxide. Rather, these scavengers react with IsoLG faster than IsoLG is able to react with the lysyl residues of proteins or with phosphatidylethanolamines, thereby significantly reducing IsoLG adduct levels (Figure 4).

To develop IsoLG scavengers, we screened small molecule primary amines with known bioavailability for their ability to prevent formation of IsoLG-[^{3}H] lysine adducts when incubated with IsoLG and [^{3}H]lysine *in vitro* [30]. Of the screened compounds, only pyridoxamine (PM), a water soluble vitamin B_6 vitamer, was able to markedly reduce IsoLG-[^{3}H]lysine formation[30]. Subsequent structure-activity relationship studies of structural analogs of PM using 4-oxopentanal (OPA) to model the reaction of 4-ketoaldehydes, showed that the second-order reaction rate of PM with OPA was more than 2,000 times faster than that of lysine with OPA[30]. Additionally, compounds which shared the 2-methylaminophenol structure of PM including salicylamine (SAM, or also known as 2-hydroxybenzylamine, 2-HOBA), showed similar high reaction rates with OPA. Importantly, the high rate of reactivity of 2-HOBA with OPA was ablated if the 2-hydroxy moiety was methylated or shifted to the para-position (4-HOBA)[30, 32]. Modification of other structural features of PM such as alkylation of the methylhydroxy group at the 5'position of pyridoxamine did not reduce the high reactivity rate with OPA.

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A mechanism for the high reactivity of 2-aminomethylphenols with IsoLG (and other 4-ketoaldehydes such as OPA) was proposed based on these structure-activity relationships. In essence, the high reactivity of these compounds was postulated to result from two key considerations: 1) the 2-hydroxy groups serves to attract the proton from the amine group, making the amine group more basic and a better nucleophile, and 2) after the initial formation of an imine adduct with IsoLG, the 2-hydroxy group of 2-HOBA helps stabilize the 4-keto group of IsoLG (through hydrogen bonding), positioning it to react with the nitrogen group of the imine and thereby form the IsoLG-2-HOBA pyrrolidine adduct, which is the rate limiting step in pyrrole formation.

These initial studies provided three compounds, PM, 2-HOBA, and PPM, that potentially could be used as IsoLG scavengers (Figure 5A). When tested for their ability to reduce cyclooxygenase-driven IsoLG adduct formation in platelets, 2-HOBA and PPM were found to have greater efficacy than PM[31]. Both also showed greater efficacy than PM in protecting HEK293 cells against hydrogen peroxide induced cytotoxicity[31]. The greater efficacy of 2-HOBA and PPM was likely due to their hydrophobicity, allowing penetration and localization within cellular membranes where IsoLGs are formed. Based on these studies, several other hydrophobic 2-aminomethylphenol compounds have been generated and shown efficacious in reducing IsoLG adducts in cells[31, 33]. In contrast, closely related phenolic compounds such as 4-HOBA (described earlier in this review), N-methyl-2-HOBA, and 5'-O-pentylpyridoxine (PPO) (Figure 5B), which are not effective dicarbonyl scavengers *in vitro* also fail to reduce IsoLG adducts in proteins or cells[18, 21]. Use of such compounds are important controls to rule out nonspecific antioxidant-like effects of 2-aminomethylphenols.

While 2-HOBA and PPM are often referred to as IsoLG scavengers, their proposed mechanisms suggest that they should be effective against other 1,4-dicarbonyls (such as 4-oxononenal, 4-oxopentanal, and succinaldehyde) as well as against 1,3-dicarbonyls (such as such as MDA). This mechanism also predicts that 2-aminomethylphenols should not be effective scavengers of 4-hydroxynonenal and other α,β-unsaturated carbonyls. Subsequent studies demonstrated that while 2-HOBA and PPM most effectively scavenge IsoLG during conditions of lipid peroxidation, they also effectively scavenge MDA[34], but not HNE[30]. Based on these results, 2-HOBA, PPM, and their analogs are best described as dicarbonyl scavengers.

The primary value of dicarbonyl scavengers is their ability to be used in cellular assays and *in vivo* conditions where oxidative injury occurs[35]. A number of studies show that these scavengers not only can protect cells or tissues from dicarbonyl-induced damage but also that these scavengers have reasonable bioavailability and do not show signs of toxicity at doses that achieve efficacy[36, 37]. For instance, 2-HOBA has been demonstrated to protect sodium channel function from inhibition by tert-butyl hydroperoxide[38] and to prevent formation of preamyloid oligomers in rapidly paced atrial cells[39]. Initial pharmacokinetic studies showed that 2-HOBA was orally bioavailable with a half-life of 62 minutes[40]. Other dicarbonyl scavengers including PPM and analogs such as PPO and 4-HOBA are also orally bioavailable (unpublished studies). Dose studies showed that up to 1 g/L of 2-HOBA in drinking water provided plasma and tissue concentrations in the range required for

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effectively scavenging IsoLG without toxicity[40]. Administration of 1 g/L 2-HOBA to mice expressing human apoE4, a model of Alzheimer's disease, protected against age-induced loss of working spatial memory[41]. As discussed in their article in this issue, Kirabo et al. have demonstrated the efficacy of 2-HOBA and a number of other dicarbonyl scavengers in a variety of models of hypertension[18]. A recent pre-clinical study demonstrated that short-term (28 day) oral administration of 2-HOBA at levels up to 1000mg/kg body weight did not cause adverse effects in rodents[42]. Additional studies are needed to evaluate long term effects, but the use of 2-HOBA has recently entered clinical trials.

Role of IsoLG modification of plasma lipoproteins in development of cardiovascular disease

In atherosclerosis, lipid modification of low density lipoprotein (LDL) triggers the uptake of LDL by macrophages which transforms to macrophage foam cells. Hoppe et al. showed that modification of LDL by IsoLG led to its uptake and degradation by macrophages[43]. This is similar to what is seen with MDA[44] or HNE[45] modification but at much lower concentrations.

While these studies suggest that IsoLG could contribute to atherosclerosis via modifying LDL, a key finding by Salomon et al. led us to consider if IsoLG might contribute by other mechanisms. They showed that only about 20% of IsoLG protein adducts isolated from plasma were associated with LDL[17]. Combining this finding with our subsequent finding that IsoLG adducts are increased in HDL of patients with familial hypercholesterolemia[21] led us to examine the role of IsoLG modification in altering HDL function.

HDL, known in the lay press as 'good cholesterol,' protects against atherosclerosis through diverse functions, including reverse cholesterol transport of cholesterol from tissues such as the aortic wall to the liver for metabolism and excretion, and the inhibition of inflammatory response of macrophages to stimulus like bacterial endotoxins[46]. Both these functions are thought to be primarily driven by ApoAI[47, 48], the major apolipoprotein of HDL (accounting for about 70% of its protein mass). In diabetics, increasing myeloperoxidase levels correlate with increased atheroma volume[49]. Incubation of HDL with myeloperoxidase leads to oxidative modifications of ApoAI and a dramatic reduction in the capacity of HDL to carry out reverse cholesterol transport[50–52]. We have found that incubation of HDL with myeloperoxidase also leads to modification of both phosphatidylethanolamine[22] and protein[21] by IsoLG and leads to ApoAI protein crosslinking[21]. Co-incubation of myeloperoxidase HDL complexes with the dicarbonyl scavenger PPM almost completely blocks IsoLG modification and crosslinking of ApoAI in HDL, whereas co-incubation with PPO, the inactive analog of PPM, has no effect on IsoLG modification or crosslinking. These findings demonstrate that IsoLG is produced downstream of an oxidative enzyme known to contribute to oxidative modification of HDL within the atherosclerotic lesion[51].

To directly assess the impact of IsoLG modification on HDL function, we incubated HDL with a range of IsoLG concentrations that produced levels of IsoLG protein adducts matching those found in patients with hypercholesterolemia or when myeloperoxidase is incubated with HDL (between 0.1 to 3 molar equivalence of IsoLG to ApoAI, respectively). We then assessed the effects of IsoLG modification on HDL functions such as its capacity to

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inhibit lipopolysaccharide (LPS)-induced macrophage inflammation, its exchangeability, and its macrophage cholesterol efflux capacity. While unmodified HDL inhibited LPS-induced cytokine release from primary murine macrophages, HDL modified with 0.1 molar equivalence of IsoLG not only completely abolishes its ability to inhibit inflammation but synergizes with LPS to produce a much greater proinflammatory cytokine expression.

This synergistic pro-inflammatory response is in contrast to the effects of PGE_2, which is produced by macrophages in response to LPS, but that suppressing macrophage activation in part by inhibiting both MyD88-dependent[53–56] and MyD88-independent[57] cytokine expression. The inhibitory effects of PGE_2 appear to be mediated by G protein-coupled receptors EP2 and/or EP4[55–60]. Recently, PGE_2 was shown to down-regulate the expression of COX-2 by increasing the expression of dual specificity phosphatase 1 (DUSP1), decreasing activity of MAPK p38, and enhancing the function of tristetraprolin [60]. Since COX-2 in macrophages potentially produces both PGE_2 and IsoLG, which appear to exert antagonistic effects, future studies are needed to understand how signaling downstream of these two compounds interact to regulate macrophage function. The signaling pathways by which IsoLG-modified HDL synergizes with LPS to further induce macrophage inflammation is not known and is currently under exploration.

We also found that modification of HDL with 1 molar equivalence or greater of IsoLG markedly reduced the exchangeability of ApoAI from HDL (termed 'ApoAI-HDL exchange'[61]), a critical step in the ability of HDL to carry out reverse cholesterol transport functions. Not surprisingly, IsoLG modification markedly reduced the capacity of HDL to efflux [^{3}H]-cholesterol from macrophages, a surrogate measure in cultured cells for *in vivo* reverse cholesterol transport. Interestingly, these modifications by IsoLG correlate with greater ApoAI protein crosslinking and the generation of a subpopulation of HDL of markedly increased diameter. Finally, we found that co-incubation of scavenger PPM was able to prevent IsoLG-induced protein crosslinking and the various described HDL dysfunctions.

Thus, modification of HDL *in vitro* with physiological relevant concentrations of IsoLG ablates its key anti-atherosclerotic functions (Figure 6), supporting the notion that the modification of HDL by IsoLG seen *in vivo* may play a key role in loss of HDL function and the development of atherosclerosis and cardiovascular disease.

Conclusions & Future Directions

The development of methods to detect and analyze proteins or lipids modified by IsoLG, as well as the development of dicarbonyl scavengers to prevent modification has given rise to a number of studies *in vitro* and *in vivo* that support the notion that IsoLG adducts contribute to atherosclerosis and diseases related to increased oxidative stress. Future studies are needed to determine the efficacy of dicarbonyl scavengers in related models of disease where IsoLG adducts have been shown to be elevated. For instance, recent studies showed that IsoLG adducts formed by macrophages isolated from adipose tissues of mice promote pathogenic T-cell responses that may be important in insulin resistance and diabetes[62], so studies are needed to examine whether dicarbonyl scavengers might to useful here. Future

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studies are also needed to elucidate the precise mechanisms whereby IsoLG modification exerts its effects. While it is easy to understand how IsoLG modification of enzymes at active site lysines or by crosslinking could lead to loss of enzyme function, IsoLG modification clearly exerts gain-of-function effects as well. One potential mechanism for such gain-of-function effects are that IsoLG modified proteins or PE act as ligands for receptors or otherwise activate cellular signaling. For example, IsoLG-PE adducts induce their inflammatory response in macrophages in part by activating the receptor for advanced glycation endproducts [22]. Dissecting the precise signaling processes whereby IsoLG adducts exert their effects may identify additional molecular targets for drug development such as previously orphan receptors. Targeting IsoLG induced signaling pathways in combination with administrating dicarbonyl scavengers might prove especially beneficial. Future studies are also needed to examine whether specifically targeting dicarbonyl scavengers to likely sites of IsoLG formation could enhance their efficacy. For instance, mitochondria are well-known sites of ROS formation and IsoLGs induce mitochondrial dysfunction[63], so that development of mitochondria-targeted scavengers might be especially valuable. Finally, future studies are needed to determine mechanism of metabolism and clearance of IsoLG modified proteins and PE *in vivo*. Enhancing catabolism of these modified macromolecules should reduce their levels and therefore potentially reduce disease. In summary, while studies to date have revealed important insights into the role of IsoLGs in cardiovascular disease and potential interventions, much work remains to be done to fully understand the mechanisms that lead to formation of IsoLG, the consequences of IsoLG modification, and the optimal strategies to prevent their adverse effects.

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Highlights:

- Isolevuglandins form stable pyrrole adducts with the lysyl residues of proteins
- Levels of proteins modified by isolevuglandins are increased by cardiovascular risk factors.
- Modification of HDL by isolevuglandin inhibits its protective functions.
- Dicarbonyl scavengers block myeloperoxidase induced isolevuglandin modification of HDL.

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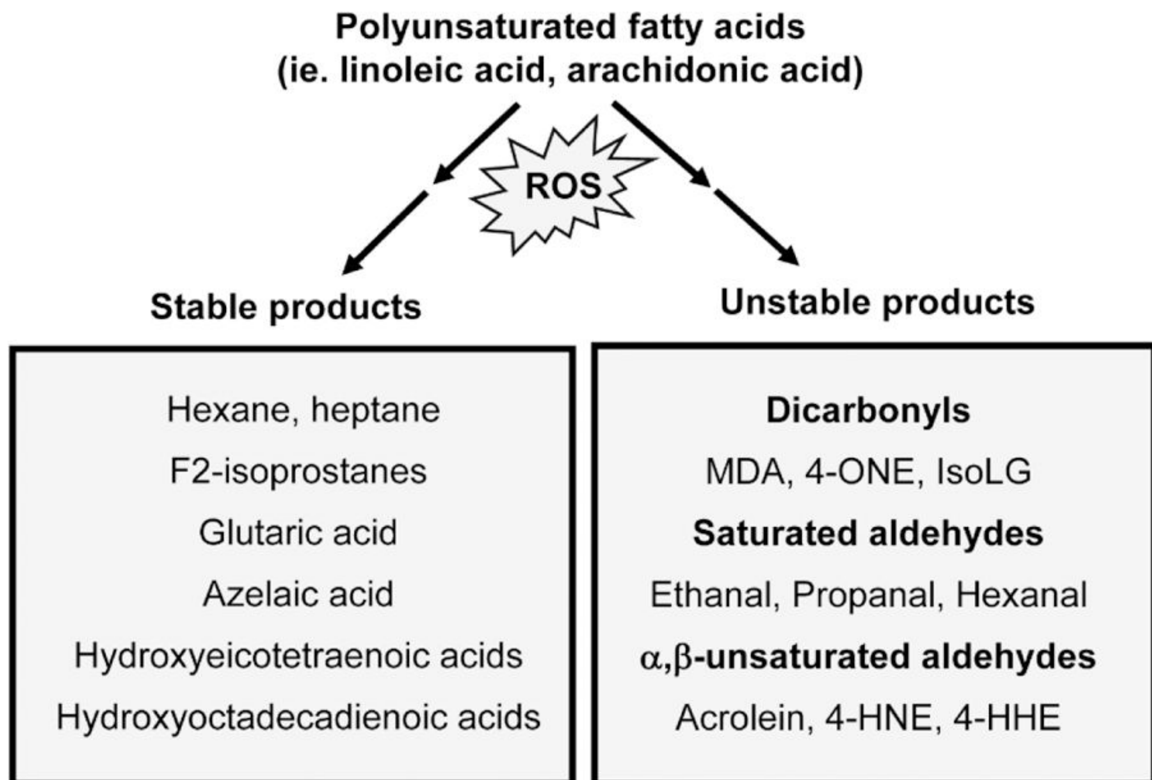

Figure 1.

Peroxidation of polyunsaturated fatty acids (PUFAs) such as linoleic acid and arachidonic acid by ROS generates a variety of stable and reactive products.

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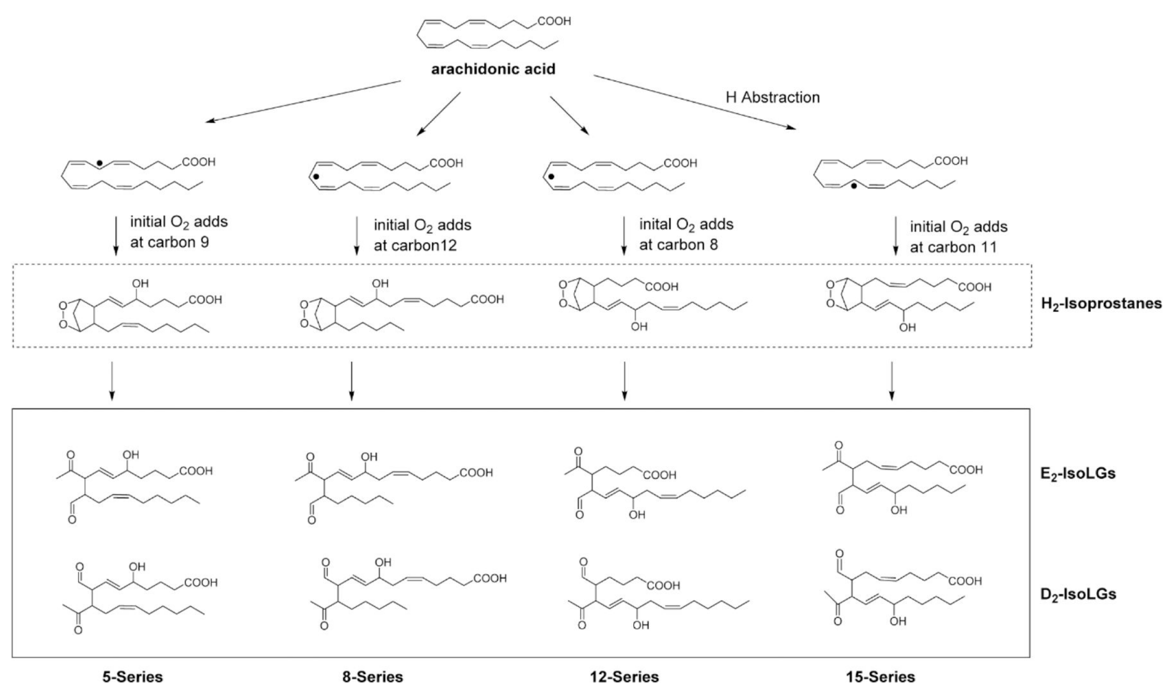

Figure 2. Formation of isolevuglandins (IsoLGs) from arachidonic acid via H_2-isoprostane intermediates.

Radicals such as the hydroxyl or a lipid peroxyl radical can abstract a hydrogen from any one of three potential positions on a molecule of arachidonic acid to form a lipid radical. Because these carbon centered radicals form between two double bonds, the resulting electron is conjugated across the five adjacent carbons. Depending on at which position that molecular oxygen (a diradical) adds to these conjugated carbon centered radical, any one of four different regioisomers of the bicyclic endoperoxides (H_2-isoprostanes) can form. Each of these regioisomers can give rise to two 4-ketoaldehyde regioisomers (D_2-and E_2-IsoLGs), generating a family of eight IsoLG regioisomers (and 64 stereoisomers since each regioisomer has 3 chiral carbons). These 4-ketoaldehydes then spontaneously react with primary amines such as lysyl residues of proteins or phosphatidylethanolamines.

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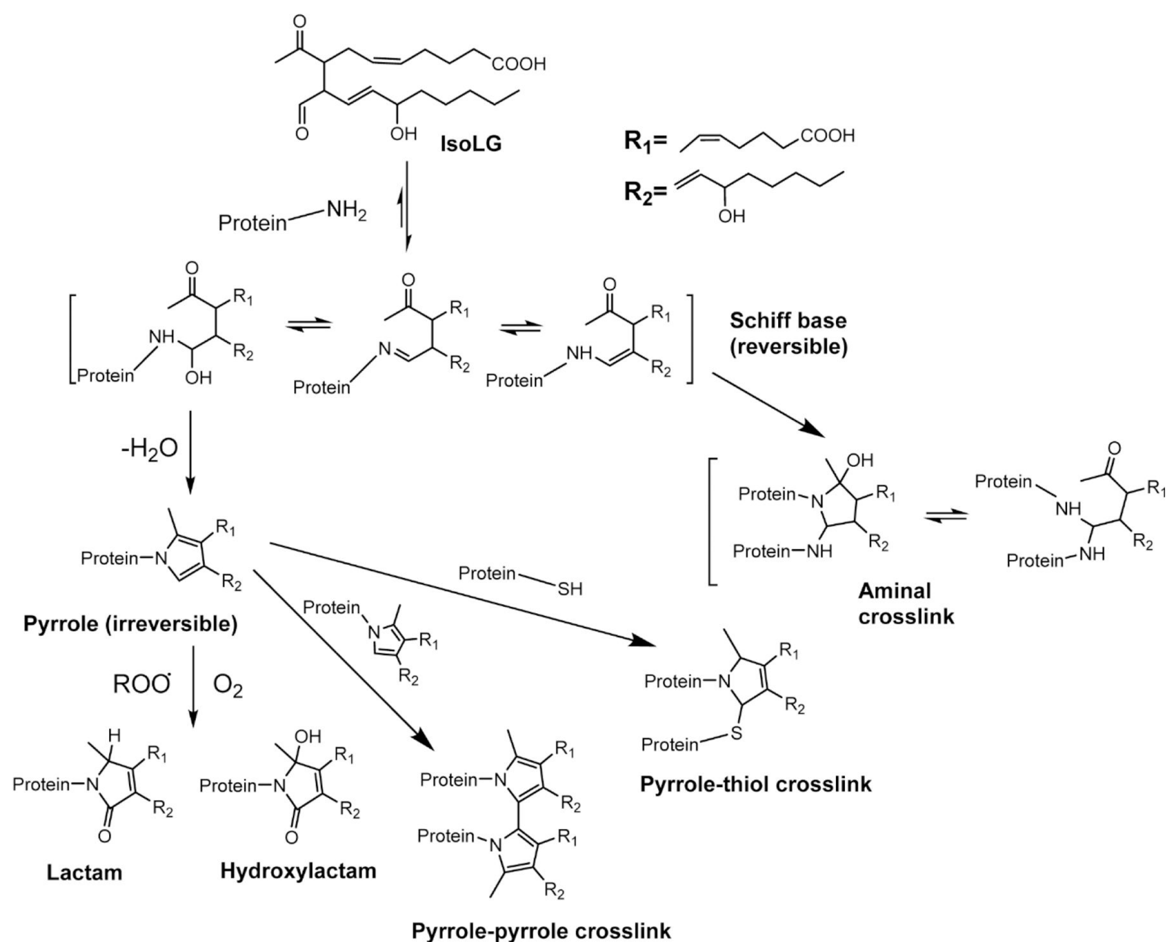

Figure 3. Mechanism of IsoLG-adduct formation.

The aldehyde group of IsoLG spontaneously reacts with primary amines to form a highly reversible imine adduct (Schiff base). The 4-keto moiety of IsoLG subsequently reacts with the nitrogen of the imine, generating a dihydropyrrolidine adduct which then loses two water molecules to form the essentially irreversible pyrrole adduct. Under oxidizing conditions, this pyrrole

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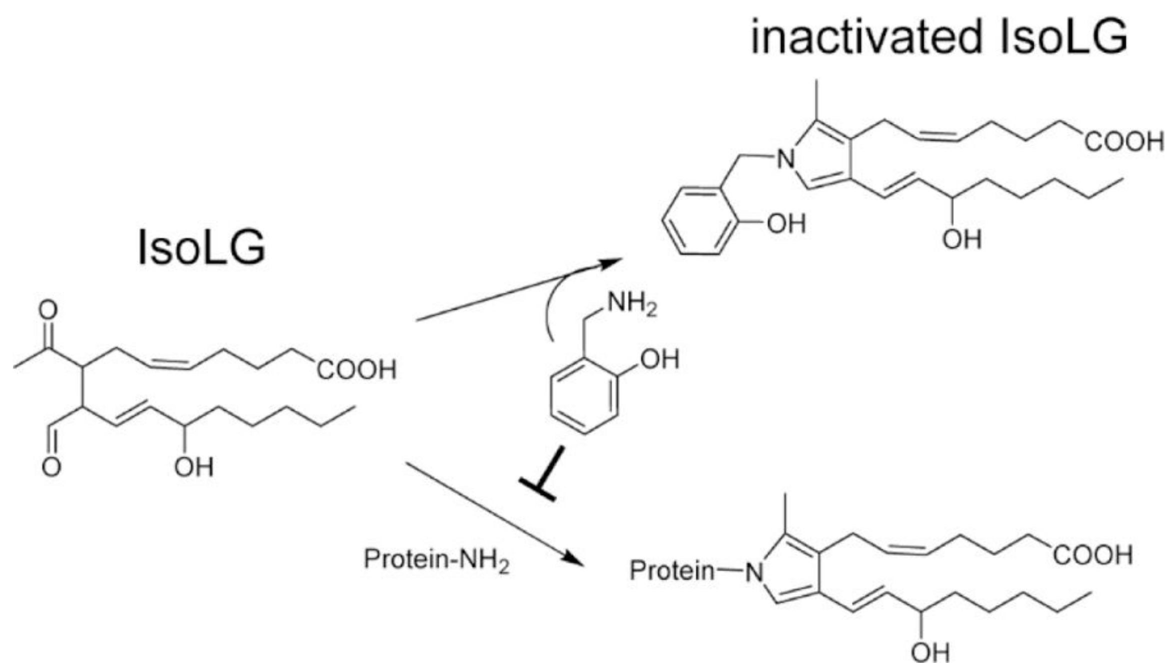

Figure 4. Scavenging of IsoLG by 2-aminomethyphenols.

The reaction of 2-HOBA with IsoLG forms a stable adduct that is poorly reactive, thereby preventing IsoLG from reacting with proteins or phosphatidylethanolamine.

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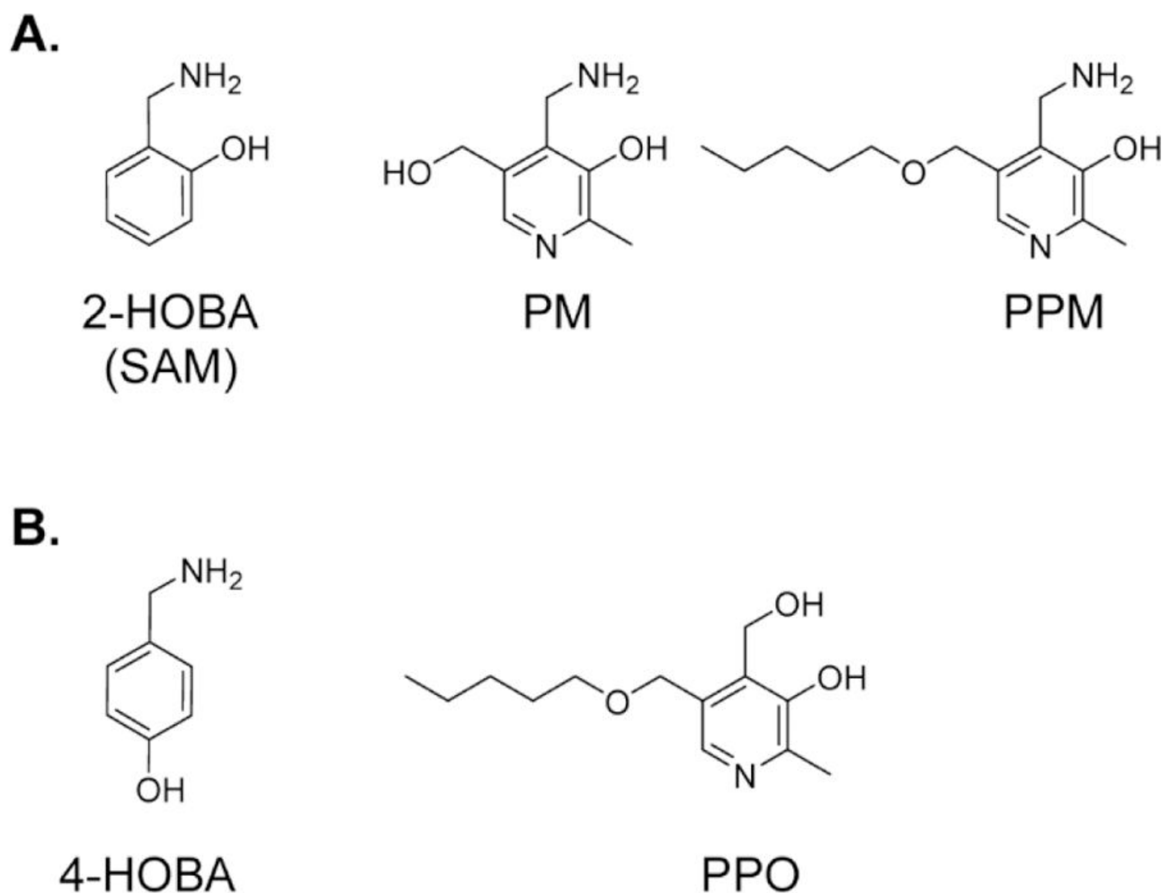

Figure 5. Dicarbonyl scavengers and their inactive analogs.

A. Notable small molecule primary amines that act as IsoLG scavengers include 2-hydroxybenzylamine (2-HOBA or SAM), pyridoxamine (PM), and 5’-O-pentyl-pyridoxamine (PPM). **B.** Analogues that are not effective scavengers include 4-hydroxybenzylamine (4-HOBA) and 5’-O-pentyl-pyridoxine (PPO).

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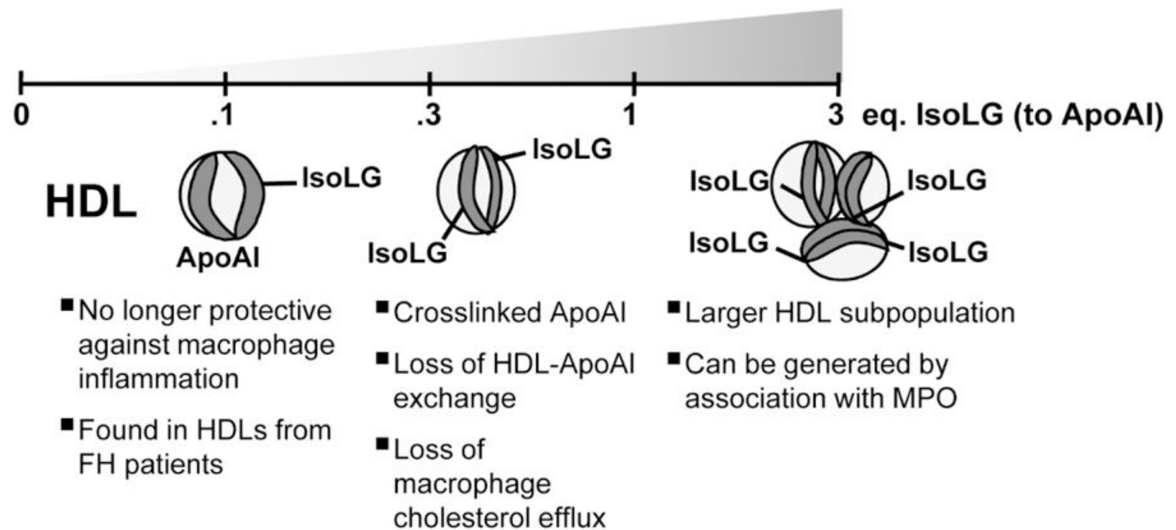

Figure 6. Effects of IsoLG modification on HDL functions.

HDL isolated by density gradient ultracentrifugation was modified with a range of IsoLG (0.1–3 molar equivalence to ApoAI) which produces adduct levels matching those found in patients with familial hypercholesterolemia or by myeloperoxidase oxidation, respectively. At the lower range of 0.1 eq, IsoLG-modified HDL is no longer protective against LPS-induced macrophage inflammation. Approaching 1 eq. modification, IsoLG-modified HDL contains crosslinked ApoAI which loses its ability to exchange and consequently has lower macrophage cholesterol efflux capacity. At greater than 1 eq., IsoLG-modification yields an HDL subpopulation with greater diameter.