**Lipid Inflammatory Mediators in Diabetic Vascular Disease**

Rama Natarajan, Jerry L. Nadler

**Abstract**—Type 2 diabetes is associated with significantly accelerated rates of macrovascular complications such as atherosclerosis. Emerging evidence now indicates that atherosclerosis is an inflammatory disease and that certain inflammatory markers may be key predictors of diabetic atherosclerosis. Proinflammatory cytokines and cellular adhesion molecules expressed by vascular and blood cells during stimulation by growth factors and cytokines seem to play major roles in the pathophysiology of atherosclerosis and diabetic vascular complications. However, more recently, data suggest that inflammatory responses can also be elicited by smaller oxidized lipids that are components of atherogenic oxidized low-density lipoprotein or products of phospholipase activation and arachidonic acid metabolism. These include oxidized lipids of the lipoxygenase and cyclooxygenase pathways of arachidonic acid and linoleic acid metabolism. These lipids have potent growth, vasoactive, chemotactic, oxidative, and proinflammatory properties in vascular smooth muscle cells, endothelial cells, and monocytes. Cellular and animal models indicate that these enzymes are induced under diabetic conditions, have proatherogenic effects, and also mediate the actions of growth factors and cytokines. This review highlights the roles of the inflammatory cyclooxygenase and 12/15-lipoxygenase pathways in the pathogenesis of diabetic vascular disease.

**Key Words:** lipoxygenase ■ diabetes ■ diabetes complications ■ inflammation ■ lipids

Diabetes is associated with significantly accelerated rates of cardiovascular complications such as atherosclerosis and hypertension. In particular, type 2 diabetes is associated with 2- to 4-fold increase in coronary artery disease. This has been attributed to the clustering of several risk factors, including insulin resistance, hypertension, obesity, and dyslipidemia. Multiple mechanisms contribute to vascular and arterial disease in the diabetic population. Basic biochemical mechanisms have been described by which hyperglycemia-induced oxidant stress activates several downstream signals that mediate diabetic complications. Furthermore, advanced glycation end products formed by glucose-induced modification of proteins can act via their receptors such as RAGE and induce cellular oxidant stress, inflammation, and vascular dysfunction in diabetes. Recent evidence from laboratory and clinical studies demonstrates that diabetic atherosclerosis is not simply a disease of hyperlipidemia but also an inflammatory disorder involving multiple mediators such as C-reactive protein, cytokines such as tumor necrosis factor alpha, and interleukin-6 (IL-6). A recent gene profiling study showed that high glucose treatment of monocytes leads to increased expression of multiple inflammatory cytokines, chemokines, and related factors, many of which are regulated by the proinflammatory transcription factor, nuclear factor-kappa B (NF-κB). The recognition now that highly effective antidiabetic agents, such as thiazolidinedio-
Figure 1. Metabolism of arachidonic acid. The cellular actions of growth factors, cytokines, and other agonists lead to the activation phospholipases and thereby release arachidonic acid. Arachidonic acid can then be metabolized by the cyclooxygenase, lipoxygenase, and cytochrome P-450 enzymes to various bioactive molecules. Note that certain LO enzymes, including 12- and 15-LO, can also react with other fatty acid substrates, such as linoleic acid, to yield additional products.

nes, and lipid-lowering agents, such as statins, possess antiinflammatory properties underscores the major role played by inflammatory mediators in the cardiovascular complications of diabetes. However, although the actions of inflammatory peptide growth factors, cytokines, and acute phase reactants have been fairly well studied, much less is known about the actions of the oxidized lipids and eicosanoids generated by these factors during cellular activation. These oxidized lipids, generated by the action of the lipoxygenase and cyclooxygenase enzymes, are produced under diabetic conditions and have potent proatherogenic effects in vessel wall cells. This review highlights the role of these pathways and their lipid products in the pathogenesis of diabetic vascular disease.

The Cyclooxygenase and Lipoxygenase Pathways
When growth factors and cytokines bind to their cell surface receptors, they can activate several phospholipases, which act on membrane phospholipids to release arachidonic acid, a precursor for several eicosanoids with potent biological effects.16,17 Arachidonic acid can be metabolized by 3 major oxidative pathways: the cyclooxygenase (COX) pathway that forms prostaglandins; the lipoxygenase (LO) pathway, which forms hydroxyeicosatetraenoic acids (HETEs) and leukotrienes; and thirdly, the cytochrome P-450 monooxygenase forms hydroxyeicosatetraenoic acids (HETEs) and leukotrienes. Proinflammatory leukotrienes have been implicated in the pathogenesis of atherosclerosis, but very little is known regarding their role in diabetes. The 12- and 15-LOs can form 12(S)- and 15(S)-HETEs from arachidonic acid. The production of 12(S)- and 15(S)-HETE has been shown in several vascular tissues and cells, including cultured vascular smooth muscle cells (VSMC), endothelial cells, and monocytes. LO products may play important roles in the pathogenesis of hypertension, atherosclerosis, and diabetes, as discussed more in detail later in the review. Functionally distinct isoforms of 12-LO have been cloned, including platelet, leukocyte, and epidermal 12-LOs.31–37 Human and rabbit 15-LOs and the leukocyte 12-LO have high homology and are classified as 12/15-LOs because they can form both 12(S)-HETE and 15(S)-HETE from arachidonic acid via their hydroperoxy precursors and mainly hydroperoxycatadecadienoic acids [13(S)-and 9(S)-HPODE] and hydroxycatadecadienoic acids from linoleic acid.31,38 The 12/15-LO has been detected in porcine leukocytes,34 VSMC,39 endothelial cells,40–42 and in several rat and mouse tissues.43–45

The Cyclooxygenase Pathway in Diabetic Vascular Disease
COX-2 and its proinflammatory products have been implicated in the pathogenesis of several inflammatory diseases including atherosclerosis because COX-2 products such as PGE2 and thromboxane have potent proinflammatory and vasoconstrictor properties.20–24,46 Furthermore, augmented expression of COX-2 was noted in atherosclerotic lesions,47 and COX-2 could promote lesion formation in low-density lipoprotein (LDL) receptor-deficient mice.48 Because COX-2 inhibitors also block formation of the protective prostacyclin, it also produces the potent inflammatory prostaglandin, PGE2.21–24

The lipoxigenase (LOs) are mainly classified as 5-, 8-, 12- or 15-LO, based on their ability to insert molecular oxygen at the corresponding carbon position of arachidonic acid (Figure 1).31–33 The 5-LO pathway leads to the formation of 5(S)-HETE and leukotrienes. Proinflammatory leukotrienes have been implicated in the pathogenesis of atherosclerosis, but very little is known regarding their role in diabetes. The 12- and 15-LOs can form 12(S)- and 15(S)-HETEs from arachidonic acid. The production of 12(S)- and 15(S)-HETE has been shown in several vascular tissues and cells, including cultured vascular smooth muscle cells (VSMC), endothelial cells, and monocytes. LO products may play important roles in the pathogenesis of hypertension, atherosclerosis, and diabetes, as discussed more in detail later in the review. Functionally distinct isoforms of 12-LO have been cloned, including platelet, leukocyte, and epidermal 12-LOs.31–37 Human and rabbit 15-LOs and the leukocyte 12-LO have high homology and are classified as 12/15-LOs because they can form both 12(S)-HETE and 15(S)-HETE from arachidonic acid via their hydroperoxy precursors and mainly hydroperoxycatadecadienoic acids [13(S)-and 9(S)-HPODE] and hydroxycatadecadienoic acids from linoleic acid.31,38 The 12/15-LO has been detected in porcine leukocytes,34 VSMC,39 endothelial cells,40–42 and in several rat and mouse tissues.43–45
diabetic conditions is only now becoming evident. In endothelial cells, high-glucose (HG) treatment increased COX-2 expression and decreased nitric oxide availability.\textsuperscript{50} Very recently, COX-2 activity and expression were shown to be upregulated by high glucose as well as ligands of the receptor for advanced glycation end products (RAGE) in monocytes, and this appeared to be primarily mediated by NF-\kappaB activation.\textsuperscript{51,52} Increased oxidant stress and protein kinase C activation under diabetic conditions could be contributory factors. Interestingly, COX-2 expression was also markedly increased in monocytes from diabetic patients.\textsuperscript{51,52} Furthermore, new data show that diabetic conditions can lead to chromatin remodeling and histone acetylation at the COX-2 gene promoter at NF-\kappaB binding sites.\textsuperscript{53} COX-2 also seemed to mediate monocyte adhesive interactions.\textsuperscript{54} A recent report demonstrated that in humans, RAGE overexpression is associated with enhanced inflammatory reactions and COX-2 expression in diabetic plaque macrophages, and that this effect could also contribute to plaque destabilization by inducing metalloproteinase expression.\textsuperscript{54} There was a significant correlation between plasma levels of hemoglobin A1c and RAGE and COX-2 expression. These results suggest that apart from its documented role in pancreatic islet dysfunction, COX-2 may be a key inflammatory mediator in the pathogenesis of diabetic atherosclerosis. Thus, the diabetic state can increase COX-2 expression and activity in vascular cells and monocytes and thereby aggravate downstream inflammatory and vascular events. It is also possible that 12/15-LO activation can increase COX-2 transcription based on studies in pancreatic islet beta cells.\textsuperscript{55}

The Lipoxigenase Pathway in Atherosclerosis, Restenosis, Diabetes, and Insulin Resistance

LO enzymes and their products, namely HETEs and hydroxycycloacadienoic acids, have been implicated in the pathogenesis of atherosclerosis. The 12/15-LO enzyme can mediate the oxidative modification of low-density lipoprotein (LDL) to the atherogenic oxidized LDL.\textsuperscript{56,57} Furthermore, angiotensin II (All) could increase macrophage-mediated modification of LDL via the 12/15-LO pathway.\textsuperscript{58} Animal models have demonstrated the key role of the LO pathway in the pathogenesis of atherosclerosis and restenosis. Overexpression of 15-LO in the vascular endothelium could accelerate atherosclerosis in LDL receptor-deficient mice.\textsuperscript{59} Leukocyte-type 12/15-LO mRNA and protein were observed in porcine atherosclerotic lesions, which were greatly augmented in diabetic and hyperlipemic pigs displaying accelerated atherosclerosis.\textsuperscript{60} LO activation may also play a role in neointimal thickening associated with restenosis because there was a marked increase in 12/15-LO expression in balloon-injured rat carotid arteries relative to uninjured. Furthermore, pretreatment with a ribozyme targeted to rat 12/15-LO could significantly reduce neointimal thickening in this rat model.\textsuperscript{61} Convincing evidence supporting a pathological role for leukocyte 12/15-LO in atherosclerosis comes from recent reports showing marked decrease in atherosclerosis in apo E\textsuperscript{−/−} mice and LDLR\textsuperscript{−/−} that were cross-bred with leukocyte 12/15-LO\textsuperscript{−/−} mice.\textsuperscript{62,63} Furthermore, a novel inflammatory link was suggested because the macrophages from 12/15-LO\textsuperscript{−/−} mice had a selective defect in lipopolysaccharide-induced IL-12 synthesis.\textsuperscript{64} An interesting genetic study suggests that 5-LO may be an important proatherogenic gene locus.\textsuperscript{65} Also, 5-LO was abundantly expressed in atherosclerotic lesions,\textsuperscript{66} and it has been suggested that 5-LO may mediate specific stages of atherosclerosis.\textsuperscript{67} However, the role of 5-LO in diabetic vascular disease is not yet known. Overall, available evidence suggests that the LOs can contribute to the pathology of atherosclerosis and diabetic vascular disease by virtue of their capacity to oxidize LDL, to induce growth and inflammatory events, and by being in an atherogenic gene locus. The relative importance of the different LOs in this regard is not yet clear. Because the 12/15-LO pathway can be upregulated by hyperglycemia, growth factors, and cytokines, it is likely that it can augment diabetic atherosclerosis and vice versa, thereby setting off a vicious loop of events.

HG culture enhanced 12/15-LO pathway activation and expression in VSMC\textsuperscript{69} and endothelial cells.\textsuperscript{42} Furthermore, All-induced 12/15-LO activity in VSMC was greater in HG relative to normal glucose.\textsuperscript{39} Apart from All, 12/15-LO activity and expression in VSMC could also be potently upregulated by platelet-derived growth factor (PDGF) BB and by cytokines such as IL-1, IL-4, and IL-8 in VSMC.\textsuperscript{68,69} The 15-LO expression was induced in monocytes and endothelial cells by IL-4 or IL-13,\textsuperscript{41,70}72 Thus, 12/15-LO in vascular and mononuclear cells can be induced by diabetic conditions, growth factors, and cytokines, and may contribute to their biological and atherogenic effects.

The LO pathway may therefore play a role in the cardiovascular complications associated with diabetes. Endothelial cells and VSMC cultured under hyperglycemic conditions produced increased amounts of HETEs.\textsuperscript{79,82,73} Furthermore, HG-induced adhesion of monocytes to endothelial cells could be mediated by the LO pathway.\textsuperscript{42,74} The 12/15-LO products also appear to mediate minimally modified LDL-induced monocyte binding to endothelial cells.\textsuperscript{75} LO products have potent chemotactic and hypertrophic effects in VSMC.\textsuperscript{76,78} The hypertrophic effects of 12(S)-HETE in VSMC were markedly enhanced under HG culture conditions in a manner similar to those of angiotensin II.\textsuperscript{77} There is now considerable evidence to support a role for 12/15-LO in promoting the development of diabetes and atherosclerosis. Bleich et al noted that 12/15-LO−deficient mice were resistant to the development of diabetes.\textsuperscript{79} In vivo relevance of 12/15-LO in human diabetes was suggested by a study demonstrating increased urinary excretion of 12(S)-HETE in diabetic subjects compared with matched nondiabetic controls.\textsuperscript{80} Interestingly, these diabetic subjects also had decreased urinary prostacyclin levels, suggesting a potential shunting into the 12/15-LO pathway. Recently, increased 12/15-LO expression was noted in a swine model of hyperlipidemia and diabetes-induced accelerated atherosclerosis.\textsuperscript{60} Diabetes and hyperlipidemia alone increased both monocyte oxidant stress and 12/15-LO expression in arteries, but the combination of these 2 risk factors in this swine model led to not only a marked acceleration of atherosclerosis but also a synergistic increase in oxidant stress and 12/15-LO activation.\textsuperscript{60} A recent report demonstrated increased expression of 12/15-LO in urine and
endothelial cells from diabetic db/db mice. Interestingly, it was noted that the increased production of 12/15-LO products by the endothelial cells of the db/db mice was responsible for the observed increase in binding of monocytes to the endothelial cells from db/db versus those from control mice, and it was concluded that the 12/15-LO pathway is important for mediating early vascular changes and inflammatory reactions in diabetes. Taken together, these results suggest an in vivo role for leukocyte type 12/15-LO in diabetic atherosclerosis.

Emerging evidence supports a clear role of insulin resistance and diabetes in leading to accelerated cardiovascular disease. As discussed, elevated glucose and diabetes increase the expression and activity of 12/15-LO. However, fewer reports have evaluated the role of 12/15-LO in metabolic disturbances seen in the insulin resistance syndrome. Of interest are studies showing that masoprocol, a LO inhibitor, can reduce triglycerides, free fatty acids, and improve insulin action in both fructose-fed and fat-fed rat models of insulin resistance and type 2 diabetes. In addition, 12-LO products can downregulate glucose transport in VSMC. To further evaluate the effect of insulin resistance on vascular injury responses and 12/15-LO expression, we studied the effect of carotid balloon injury in lean and obese insulin-resistant Zucker rats. After injury, the intima-to-media ratio of obese Zucker rats was significantly greater than leans starting at 14 days after injury and persisting up to at least day 30. The expression of inflammatory mediators including 12/15-LO and IL-6 were markedly increased in obese compared with lean animals suggesting that vascular injury in obese Zucker rats is associated with inflammation. Increased macrophage and p-selectin staining was also seen. These studies (unpublished) indicate an exaggerated injury response in the insulin resistant obese Zucker rat model and that inflammation may play a major role in mediating neointimal growth under these conditions. In addition, 12/15-LO was one of the few genes upregulated in the pancreatic beta cell of the insulin resistant prediabetic Zucker diabetic fatty rat, thereby suggesting that 12/15-LO expression is enhanced in the prediabetic metabolic syndrome condition before frank hyperglycemia. Thus 12/15-LO may have a role in the excess cardiovascular disease seen even before diabetes is diagnosed. Because hyperglycemia alone also increases 12/15-LO expression in vascular cells, it is likely that 12/15-LO can participate in the development of type 2 diabetes and atherosclerosis, whereas the associated hyperglycemia, dyslipidemia, and insulin resistance can further augment 12/15-LO pathway activation to set off a vicious loop of inflammatory events. Furthermore, factors such as growth factors, cytokines, and advanced glycation end products, all of which are relevant to the pathogenesis of diabetes, can also upregulate the activity and expression of 12/15-LO (Figure 2).

**Lipoxygenase Products Have Growth, Chemotactic, Adhesive, and Inflammatory Effects in VSMC and Endothelial Cells**

Treatment of human aortic endothelial cells with 12(S)-HETE, but not 12(R)-HETE, increased monocyte binding to the endothelial cells, a key early step in the development of atherosclerosis. Furthermore, the 12/15-LO ribozyme blocked high-glucose–induced binding of monocytes to endothelial cells, suggesting that glucose-induced LO activation in endothelial cells may lead to endothelial activation and dysfunction. The 12(S)-HETE increased the expression of CS-1 fibronectin on endothelial cells, which could be a key mechanism for inducing monocyte adhesion. Certain LO products also increased the surface expression of key inflammatory adhesion molecules such as VCAM-1 via activation of the transcription factor, NF-κB. LO products also directly increased migration, cellular hypertrophy, and fibronectin synthesis in VSMC. Angiotensin II (AII)-induced increases in total cellular protein content of porcine VSMC were significantly attenuated by a specific LO inhibitor. Furthermore, direct addition of the 12-LO product, 12(S)-HETE, increased total cell protein content and fibronectin levels to nearly the same extent as AII. A rat 12/15-LO ribozyme could significantly inhibit HG-induced fibronectin production. Because AII and HG culture can increase the formation of LO products, it is attractive to speculate that the enhanced growth-promoting and matrix effects of the LO products formed by AII and HG are potential mechanisms for the accelerated growth of VSMC and enhanced hypertrophic effects of AII under HG conditions. In support of this, it was noted that rat VSMC and
cardiac fibroblasts stably expressing mouse 12/15-LO showed increased growth properties.\textsuperscript{78,88} In addition, pharmacological LO inhibitors, as well as the 12/15-LO ribozyme, could also significantly inhibit PDGF-induced migration of VSMC.\textsuperscript{42,68} Because PDGF can upregulate 12/15-LO,\textsuperscript{68} LO activation may mediate, at least in part, the chemotactic effects of PDGF.

LO products also have proinflammatory effects in VSMC. Thus the 12/15-LO product of linoleic acid, 13-HPODE, led to a significant increase in the activation of the redox-sensitive and inflammatory transcription factor, NF-\( \kappa \)B in VSMC.\textsuperscript{87} This was associated with increased transcription of the inflammatory adhesion molecule VCAM-1 and the potent chemokines monocyte chemoattractant protein-1 via an NF-\( \kappa \)B–dependent mechanism.\textsuperscript{87,89}

**Signal Transduction and Gene Regulation Mechanisms by Which LO Products Mediate Their Cellular Actions**

HETEs can activate certain isoforms of protein kinase C directly or indirectly by incorporating into membrane phospholipids, which then generate HETE-containing diacylglycerol species to activate protein kinase C.\textsuperscript{90} They can also activate several mitogen activated protein kinases (MAPKs) and thereby activate key transcription factors that mediate the expression of growth and inflammatory genes.\textsuperscript{78,90} In VSMC, 12(S)-HETE could lead to the transcription of the fibronectin gene, and this was regulated by the transcription factor CREB in a p38MAPK–dependent manner.\textsuperscript{90} However, the hydroperoxy LO product, 13(S)-HPODE, could increase the expression of VCAM-1 in an NF-\( \kappa \)B–dependent manner and partly via p38MAPK.\textsuperscript{97} Thus these oxidized lipids can serve as novel signal transducers, regulators, and amplifiers of gene induction by high glucose, growth factor, and cytokine actions. Interestingly, a novel role for HPODE and hydroperoxy precursor as seeding molecules responsible for LDL oxidation by artery wall cells and associated oxidative events related to the pathogenesis of atherosclerosis has been demonstrated.\textsuperscript{91} In monocytes, 9-hydroxyoctadecadienoic acid and 13-hydroxyoctadecadienoic acid (12/15-LO products of linoleic acid metabolism) induced the expression of the scavenger receptor, CD36, apparently via activation of the nuclear receptor, peroxisome proliferator activator-gamma.\textsuperscript{92} IL-4–induced 12/15-LO activation was also implicated in this process.\textsuperscript{93}

Coffey et al demonstrated that 12/15-LO can lead to the catalytic consumption of the vasodilator, nitric oxide, and prevent nitric oxide–mediated soluble guanylate cyclase activation.\textsuperscript{94} This suggests that 12/15-LO may mediate the pathology of vascular diseases such as atherosclerosis, hypertension, and diabetes not only by the bioactivity of their lipid products but also by limiting the availability of nitric oxide in the vessel wall. Reactive oxygen species generated during LO pathway activation\textsuperscript{95} may mediate growth and inflammatory effects in VSMC and endothelial cells. Conversely, high-glucose induced oxidant stress, and reactive oxygen species in diabetes can lead to the induction of 12/15-LO in VSMC and endothelial cells and promote cellular dysfunction. Recent reports show that VSMC derived from 12/15-LO\textsuperscript{-/–} mice grow slower than those derived from genetic control mice, produce much lesser amounts of superoxide, and have reduced activation of MAPKs,\textsuperscript{96} whereas endothelial cells derived from these 12/15-LO KO mice display decreased binding to monocytes compared with those from control mice.\textsuperscript{97} However, new data show that endothelial cells from 12/15-LO transgenic mice reciprocally display enhanced monocyte binding relative to those from control mice.\textsuperscript{97} Interestingly, these 12/15-LO transgenic mice also developed more atherosclerotic lesions.\textsuperscript{97} Overall, it appears that 12/15-LO can participate in an inflammatory loop with cytokines and other inflammatory genes to amplify or modulate their cellular responses and thereby accelerate the development of cardiovascular complications in diabetes.

In summary, LO and COX-2 enzymes in vascular, inflammatory, and other cells can form products with pleiotropic physiological and pathological effects. These include vasoactive, growth, adhesive, chemotactic, oxidative, and inflammatory responses, which therefore implicate them in the pathogenesis of diabetic vascular disease such as atherosclerosis and hypertension. Although COX-2–specific inhibitors are now available for clinical use, there are currently no clinically safe, pharmacologically selective, and optimally bioavailable inhibitors of 12/15-LO. Hence, the development of 12/15-LO inhibitors, including novel ribozymes, may lead to new antiinflammatory therapies for diabetic vascular complications.

**Acknowledgments**

We acknowledge grant support from the National Institutes of Health (PO1 HL55798, RO1 DK55240, RO1 DK065073, and RO1 DK58191) and the Juvenile Diabetes Research Foundation International. We thank Dr Q. Cai for his help with the manuscript.

**References**


11. Schmidt AM, Hori O, Chen J, Li JF, C randall J, Zhang J, Cao R, Yan SD, Brett J, and Stern D. Advanced glycation end products interacting with...


Lipid Inflammatory Mediators in Diabetic Vascular Disease
Rama Natarajan and Jerry L. Nadler

Arterioscler Thromb Vasc Biol. 2004;24:1542-1548; originally published online May 27, 2004;
doi: 10.1161/01.ATV.0000133606.69732.4c

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://atvb.ahajournals.org/content/24/9/1542

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/