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-
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. Arachidonic acid can be metabolized by the cyclooxygenase (COX) pathway that forms prostaglandins; the lipoxygenase (LO) pathway, which oxidizes lipids, generated by the action of the lipoxygenase, and cytochrome P-450 enzymes, to yield additional products. 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.

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. Furthermore, augmented expression of COX-2 was noted in atherosclerotic lesions, and COX-2 could promote lesion formation in low-density lipoprotein (LDL) receptor-deficient mice. Because COX-2 inhibitors also block formation of the protective prostacyclin, it also produces the potent inflammatory prostaglandin, PGE2.

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diabetic conditions is only now becoming evident. In endo-
thelial cells, high-glucose (HG) treatment increased COX-2 expression and decreased nitric oxide availability.50 Very
currently, 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-κB
activation.51,52 Increased oxidant stress and protein kinase C
activation under diabetic conditions could be contribu-
tory factors. Interestingly, COX-2 expression was also markedly
increased in monocytes from diabetic patients.51,52 Fur-
thermore, new data show that diabetic conditions can lead to
chromatin remodeling and histone acetylation at the COX-2
gene promoter at NF-κB binding sites.53 COX-2 also seemed
to mediate monocyte adhesive interactions.51 A recent report
demonstrated that in humans, RAGE overexpression is asso-
ciated 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.54 There was a signif-
ificant 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 patho-
genesis of diabetic atherosclerosis. Thus, the diabetic state
can increase COX-2 expression and activity in vascular cells
and monocytes and thereby aggravate downstream inflamma-
tory and vascular events. It is also possible that 12/15-LO
activation under diabetic conditions could be contribu-
tory factors. Recently, 12/15-LO expression in arteries, but the combination of these
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.60 A recent report
demonstrated increased expression of 12/15-LO in urine and

from 12/15-LO−/− mice had a selective defect in lipopolysac-
charide-induced IL-12 synthesis.64 An interesting genetic
study suggests that 5-LO may be an important proatherogenic
gene locus.65 Also, 5-LO was abundantly expressed in ath-
erosclerotic lesions,66 and it has been suggested that 5-LO
may mediate specific stages of atherosclerosis.67 However,
the role of 5-LO in diabetic vascular disease is not yet known.
Overall, available evidence suggests that the LOs can con-
tribute to the pathology of atherosclerosis and diabetic vas-
cular 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 VSMC69 and endothelial cells.42 Furthermore,
AI-induced 12/15-LO activity in VSMC was greater in HG
relative to normal glucose.59 Apart from AI1, 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.68,69
The 15-LO expression was induced in monocytes and endo-
thelial cells by IL-4 or IL-13.41,70–71 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 cardio-
vascular complications associated with diabetes. Endothelial
cells and VSMC cultured under hyperglycemic conditions
produced increased amounts of HETEs.72,73 Furthermore,
HG-induced adhesion of monocytes to endothelial cells could
be mediated by the LO pathway.72,74 The 12/15-LO products
also appear to mediate minimally modified LDL-induced
monocyte binding to endothelial cells.73 LO products have
potent chemotactic and hypertrophic effects in VSMC.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.72 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.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 sub-
jects compared with matched nondiabetic controls.80 Inter-
estingly, 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.80 Diabetes and hyperlip-
idemia 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.80 A recent report
demonstrated increased expression of 12/15-LO in urine and

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

LO enzymes and their products, namely HETEs and hy-
droxycatadecadienoic 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.56–57 Furthermore,
angiotensin II (AII) could increase macrophage-mediated
modification of LDL via the 12/15-LO pathway.58 Animal
models have demonstrated the key role of the LO pathway in
the pathogenesis of atherosclerosis and restenosis. Overex-
pression of 15-LO in the vascular endothelium could accel-
erate atherosclerosis in LDL receptor-deficient mice.59
Leukocyte-type 12/15-LO mRNA and protein were observed
in porcine atherosclerotic lesions, which were greatly aug-
mented in diabetic and hyperlipemic pigs displaying acceler-
ated atherosclerosis.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.61 Convincing evidence supporting a patholo-
gical role for leukocyte 12/15-LO in atherosclerosis comes
from recent reports showing marked decrease in atheroscle-
rosis in apo E−/− mice and LDLR−/− that were cross-bred with
leukocyte 12/15-LO−/− mice.62,63 Furthermore, a novel
inflammatory link was suggested because the macrophages
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 increased 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.

**Figure 2.** Actions of 12/15-LO in the vessel wall. Induction of 12/15-LO and its products in endothelial cells by factors such as HG and AGEs can lead to oxidant stress, release of chemokines, activation of monocyte integrins, and key endothelial adhesive molecules and thereby lead to endothelial dysfunction, monocyte activation, and adhesion. In VSMC, similarly, 12/15-LO and its products can induce oxidant stress, adhesion molecules, extracellular matrix proteins, release of inflammatory cytokines, and chemokines, thereby leading to VSMC hypertrophy, migration, and inflammatory responses. 12/15-LO in monocyte/macrophages, endothelial cells, and VSMC can mediate LDL oxidation to oxidized LDL.
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-kB in VSMC.\textsuperscript{67} This was associated with increased transcription of the inflammatory adhesion molecule VCAM-1 and the potent chemokines monocyte chemoattractant protein-1 via an NF-kB–dependent mechanism.\textsuperscript{67,69}

**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{91} However, the hydroperoxy LO product, 13(S)-HPODE, could increase the expression of VCAM-1 in an NF-kB–dependent manner and partly via p38MAPK.\textsuperscript{91} 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 monocyes, 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–/– 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.

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Natarajan and Nadler
Diabetes, Inflammation, and Vascular Disease


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
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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