# 12-Lipoxygenases

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Lipoxygenases (LOXs) are lipid metabolizing enzymes that catalyze the di-oxygenation of polyunsaturated fatty acids to generate active eicosanoid products. 12-lipoxygenases (12-LOXs) primarily oxygenate the 12th carbon of its substrates.

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## 1. 12-LOXs in Insulin Resistance and Obesity

In obesity, tissue insulin resistance creates demand upon the β cell to enhance insulin release. T2D occurs when the demand exceeds the capacity of the β cell to meet insulin requirements, reflecting an inherent dysfunction of β cells in some individuals [1]. As macrophages accumulate in tissues (e.g., adipocytes, and islets) during obesity, they produce pro-inflammatory cytokines (e.g., TNF-α, IL-1β, and IFN-γ) that cause biochemical and physiological effects locally within the tissues and systemically, leading to insulin resistance [2]. Macrophage 12-LOXs are upregulated under conditions such as hyperglycemia and systemic inflammatory responses to promote macrophage migration and a pro-inflammatory state through the production of pro-inflammatory cytokines [3][4][5][6][7]. 12/15-LOX isoforms (encoded by human *ALOX12* and *ALOX15*) were highly expressed in adipose tissues from patients with obesity, particularly in the stromal vascular fraction (SVF), which contains the majority of inflammatory cells such as macrophages [8]. Furthermore, the addition of 12-HETE and 12-HPETE to adipocytes in culture promotes the expression of pro-inflammatory cytokines and chemokines, such as TNF-α, MCP-1, and IL-6.

When *Alox15*$^{-/-}$ mice are fed a high-fat diet (either a Western diet composed of ~45% kcal from fat or a high-fat diet composed of 60% kcal from fat), they show protection from impaired glucose and insulin resistance compared to similarly-challenged control mice [9][10]. Consistent with the reduction in insulin resistance, islets from these mice do not exhibit the adaptive hyperplasia seen in controls [9][10]. Adipose depots from dietary challenged *Alox15*$^{-/-}$ mice contain significantly less macrophage infiltration, consistent with either a potential role for 12-LOXs in macrophage migration or in adipose tissue chemokine secretion [9][10]. With regard to the latter possibility, studies from adipose-specific *Alox15* knockout mice (using aP2-Cre-driven deletion) also revealed improved glucose metabolism and reduced macrophage infiltration on a high-fat diet [11]. However, whereas the aP2-Cre transgene has been widely used to achieve pan-adipose gene deletion, *aP2* is also expressed in various tissues, including macrophages [12], and is involved in myeloid lineage differentiation during development [13].

A third key tissue that is involved in T2D pathogenesis is the islet β cell, which is also a site of activity by 12-LOXs. Deletion of murine *Alox15* in the pancreas (using Pdx1-Cre-mediated deletion) results in the loss of 12-LOXs primarily in β cells, since *Alox15* does not show substantial expression in other pancreatic cell types [14]. In these studies, pancreas-specific *Alox15* knockout mice showed protection from high-fat diet-induced glucose intolerance, with augmented adaptive islet hyperplasia and increased insulin levels [14]. These findings emphasize the potentially suppressive role of 12-LOXs and its products on β-cell function in response to insulin resistance. Taken together, studies on the perturbation of 12-LOXs in adipose, macrophages, and pancreatic islets emphasize a central role for their activity in the pathogenesis of insulin resistance and T2D.

## 2. 12-LOXs in Hepatic Inflammation and Disease

12-LOXs have been implicated in hepatic inflammation [15]. Hepatic inflammation can lead to a range of pathologies from non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) to alcoholic liver disease and ischemia-reperfusion injury. In these studies, roles of 12-LOXs encoded by both mouse *Alox12* and *Alox15* appear to be relevant. In one study, whole-body *Alox15*$^{-/-}$ mice were protected from hepatic steatosis under conditions of high-fat diet feeding. The infiltration by lymphocytes was diminished, pro-inflammatory cytokine mRNA levels (TNF-α and IFN-γ) were reduced, and there was a general reduction of chemoattractant mRNA levels, including that of *Cxcl1* and *Cxcl2/3* [16]. A potential role for 12-LOXs in NASH has also been reported. In a murine model of NASH, which involves the feeding of

methionine and choline-deficient diet (MCD) [17], one study observed increased transcription of *Alox12* in hepatocytes and upregulation of serum 12-HETE levels [18]. An upregulation in both the *Alox12* mRNA and protein levels of 12-LOX in the liver was recently observed in these mice, along with enhanced activity of 12-HETE and 15-HETE oxygenation products in the cytosol of the hepatocytes [19]. It remains to be seen, however, if deletion of *Alox12* in this model protects against the development of NASH.

Hepatic ischemia-reperfusion injury is a disease process involving ischemia-mediated hepatocellular damage that is paradoxically exacerbated by subsequent liver reperfusion [20]. Levels of 12-LOX (encoded by *Alox12*) were found to be significantly upregulated in hepatocytes during the ischemic phase, leading to the accumulation of 12-HETE and to the production of pro-inflammatory mediators TNF-α, IL-6, and Cxcl2. The production of these pro-inflammatory mediators was shown to be mediated by a signaling axis involving the 12-HETE receptor GPR31 [21]. In a separate study, it was shown that the 12-LOX–12-HETE axis was activated in liver ischemia-reperfusion injury, and its activation was further enhanced in fatty liver. In this study, inhibition of 12-LOXs by the selective small-molecule inhibitor ML355 [22] mitigated the liver damage, and studies in vitro showed that 12-HETE increased the expression of GPR31 and activated the downstream PI3K/Akt/NF-κB pathway [23].

Finally, the role of 12-LOXs have also been studied in alcohol-induced fatty liver. Chronic alcohol consumption leads to ER and oxidative stress as well as liver injury in the form of fatty liver, alcoholic hepatitis, and cirrhosis [24]. Whole-body *Alox15*$^{-/-}$ mice were protected from alcohol-induced liver disease progression via suppression of oxidative and ER stress [25]. Alcohol feeding in this study led to an increase in reactive oxygen species generation in the liver of control mice, but was significantly reduced in *Alox15*$^{-/-}$ mice. Similarly, there was a marked reduction in classic ER stress markers IRE1α, ATF4, XBP1, and CHOP in *Alox15*$^{-/-}$ mice [25]. Collectively, these studies demonstrate that the 12-HETE-GPR31 pathway is activated in the setting of inflammation from both liver ischemia-reperfusion injury and NASH, while in the setting of alcohol induced liver disease, 12-LOXs play a pathogenic role through oxidative and ER stress pathways.

## 3. 12-LOXs in Gastrointestinal Inflammation and Autoimmune Disease

Eicosanoids produced from the conversion of arachidonic acid at the cell membrane have been shown to participate in host defense in the gastrointestinal system [26]. While the role of prostaglandins has been extensively studied in the pathogenesis of inflammatory bowel disease (IBD ), recent work has demonstrated that lipoxygenase products might play a role in autoimmune-related gut inflammation [26][27].

*Alox15* and its encoded 12-LOX is expressed in the intestinal epithelium [28]. Indeed, high levels of 12-HETE have been found in colonic mucosal tissue from patients with inflammatory bowel disease by thin-layer chromatography and high-performance liquid chromatography [27][29]. The neutrophil chemoattractant hepoxilin A3 ($HXA_3$) is a downstream metabolite of 12-HPETE, and $HXA_3$ functions in polymorphonuclear leukocyte (PMN) recruitment to sites of mucosal inflammation. Using a model that has been described to study basolateral to apical PMN transepithelial migration [30], it has been demonstrated that $HXA_3$ promotes the final step of PMN recruitment to sites of inflammation by establishing a gradient across the epithelial tight junction. Inhibition of 12-LOXs by treatment with the small molecule inhibitor baicalein lead to blockage of $HXA_3$ generation and inhibition of PMN transmigration stimulated by *Salmonella typhimurium* infection [31]. Furthermore, 12-LOXs have been shown to play a pathophysiological role in an animal model of IBD. In a dextran sodium sulfate (DSS) induced colitis model that was restricted to female mice, *Alox15*$^{-/-}$ mice were robustly protected from colitis and weight loss by a mechanism involving sustained epithelial tight junction protein expression [32]. Interestingly, expression of *Alox15* was not seen in healthy mouse colon but was significantly upregulated in the inflamed colon after 8 days of DSS induced colitis. Expression was restricted to the stromal cells, which represent invading leukocytes. Inflammatory marker analysis revealed that *Alox15* deficient mice exhibited less colonic macrophage infiltration assessed by F4/80 staining and mRNA analysis of distal colon revealed increased iNOS and TNF-α expression. Colon permeability studies revealed that *Alox15* deficient mice had significantly reduced permeability and higher functional ZO-1 expression compared to the DSS-treated control mice.

Thus, products of 12-LOXs appear to play a dual role in gut inflammatory disease by affecting gut epithelial cell integrity and by promoting polymorphonuclear leukocyte migration.

## 4. 12-LOXs and Cardiovascular Disease

Vascular remodeling is an active process in which the vessel wall thickens owing to vascular smooth muscle cell (VSMC) migration, thrombosis, and proliferation, leading to the formation of a thickened neointima layer in response to elevated shear stress, pressure, or arterial injury [33]. 12-LOXs have been implicated in VSMC migration, proliferation and apoptosis

[34]. The metabolites 15-HETE and 12-HETE have both been found to act as mitogens in a MAPK dependent pathway in vascular endothelial cells and VSMCs [34][35]. Both 12-HETE and 15-HETE have been shown to promote VSMC migration via cAMP-response element-binding protein (CREB) mediated IL-6 expression [36].

Atherosclerotic lesions develop at sites of endothelial dysfunction and represent a chronic inflammatory process characterized by macrophage chemotaxis, accumulation, and foam cell formation [37]. Because of its ability to oxidize biomembranes and lipoproteins, 12-LOXs have been studied in the setting of atherogenesis. Human atherosclerotic lesions contain *ALOX15* mRNA and 12-LOX products [38][39]. 12-HETE has been shown to directly induce monocyte binding to human aortic endothelial cells [5][40], promote endothelial wall disruption [41], and to directly oxidize LDL, which contributes to foam cell formation [42][43]. In this regard, studies in which mice have been crossed with athero-susceptible backgrounds have shown protection from the development of atherosclerosis [44][45][46].

The most widely used mouse models of atherosclerosis are the *ApoE*$^{-/-}$ *and Ldlr*$^{-/-}$ mice, which both develop hypercholesterolemia [47]. It has been demonstrated that *Alox15*$^{-/-}$ deficient mice on the *ApoE* null background developed significantly reduced atherosclerotic lesions even at 1 year of age. This observation was likely due to a reduction in oxidized LDL given the reductions in plasma autoantibody titers to oxidized LDL epitopes in double knockout mice compared to controls [46]. In another study, deletion of *Alox15* in the *Ldlr*$^{-/-}$ background also lead to a significant reduction in plaque formation at 3, 9, 12, and 18 weeks of high-fat diet feeding, while the cellular content of macrophages and T cells within the plaques did not change [44]. Both studies showed that *Alox15* deficiency did not influence lipid profiles. However, a study in which *Alox15*$^{-/-}$*;Ldlr*$^{-/-}$ mice were fed a polyunsaturated fatty acid-enriched diet (10% calories as safflower oil) in which 12-LOX products are enhanced, atherosclerosis was reduced, but levels of cholesterol and triglyceride also decreased with improvement in hepatic steatosis compared to controls [48]. Therefore, 12-LOXs are involved in several steps in the pathogenesis of atherosclerosis, specifically through the promotion of LDL oxidation and induction of a pro-inflammatory state which promotes macrophage metabolic activity. Whether 12-LOXs products affect lipid profiles relevant to human disease has not been demonstrated.

## 5. 12-LOXs in Neuroinflammation and Neurodegenerative Disease

Neuroinflammation is an underlying cause of neuronal damage and brain disorders, including Alzheimer's disease (AD) and Parkinson's disease (PD) [49][50][51]. One of the main features of AD is the presence of senile plaques containing beta-amyloid peptide. 12-LOXs and 12-HETE are highly expressed in neurons of human brains [52], and 12-LOXs have been implicated in promoting neuroinflammation in both humans and mice [30]. Different mechanisms have been proposed for 12-LOXs mediated neuroinflammation, including its role in increasing oxidative stress in the neurons [52][53]. The transcription factor c-Jun is associated with neuronal apoptosis and has been shown to be active following exposure of neurons to the beta-amyloid peptide found in AD [54]. However, inhibition of 12-LOXs using anti-sense oligonucleotide strategy leads to a disruption in c-Jun dependent beta-amyloid-induced apoptosis in cortical cells [55]. Post-mortem analyses of brain sections from patients with AD show increased levels of 12-LOXs in the temporal and frontal lobes, with increased levels of both 12-HETE and 15-HETE [52]. Another study reported increased levels of 12-HETE in the cerebrospinal fluid of AD patients [56]. These studies implicate a role for 12-LOXs in the formation of beta-amyloid deposits. In a transgenic mouse model of AD-like amyloidosis in which mice develop amyloid deposits and cognitive impairment, mice were found to have a significant reduction in beta-amyloid production and deposition, as well as improvement in memory in the absence of *Alox15* [57].

Neuroinflammation is also considered as an underlying cause of the pathogenesis of Parkinson's disease [58]. Although the pathogenesis of PD has focused on the presence of Lewy bodies in dopaminergic neurons, more recent studies have implicated oxidative stress and altered protein metabolism in precipitating the risk for PD development [58][59]. The 12-LOX pathway has been implicated with the progression of Parkinson's disease through its role in oxidative and ER stress. An early finding of PD is a reduction in the level of the anti-oxidant glutathione [60], and its reduced levels lead to increased levels of nitric oxide (NO) in the neurons, which is neurotoxic [61]. An in vitro study with rat midbrain cultures showed that the NO-mediated neurotoxicity is reduced with the inhibition of 12-LOX with baicalein [62]. Moreover, studies in vitro with murine neurons show that a reduction in glutathione levels is associated with the upregulation of 12-LOX protein levels as well as 12-HETE [63].

12-LOXs and their pro-inflammatory mediators have also been implicated in nerve cell death (94) and brain ischemia [64] [65]. In a murine model of transient middle cerebral artery occlusion, levels of 12-LOX were increased in the peri-infarct region of the neurons, and expression levels of 12-HETE also increased after brain ischemia in gerbil forebrains [66]. In a mouse study, when 12-LOX was inhibited by baicalein or genetically inactivated (*Alox15*$^{-/-}$), mice were protected against transient focal ischemia [64]. A recent study showed that subarachnoid hemorrhage led to increased 12-LOX protein levels

in murine brain macrophages and promoted neuronal death. When 12-LOX was inhibited by ML351 or by genetic inactivation (*Alox15*$^{-/-}$), neuronal death was reduced, resulting in protection from brain edema and improved behavioral outcomes [67]. Treatment of mice with a novel inhibitor of 12-LOX, LOXBlock-1 reduced infarct sizes at both 24 h and 14 days post-stroke, with improved behavioral parameters. LOXBlock-1 also reduced oxidative stress in the cultured murine neuronal HT22 cells. It was observed that 12-LOX co-localized with lipids MDA2. This co-localization was also detected in the brain of two human stroke patients [65]. Indeed, plasma levels of 12-HETE were found to be elevated up to 7 days after stroke in a biomarker study that involved over 60 stroke patients [68]. Together, these data point to a critical role of 12-LOX in oxidative stress-related glutathione depletion in neuronal cell death relevant to human disease.

## 6. 12-LOXs in Pulmonary Inflammation and Disease

Pulmonary inflammation can be caused by infectious or non-infectious agents. Acute pulmonary inflammation leads to immune cell infiltration, mucus production, vascular leak into the airways, and epithelial cell damage. Unregulated inflammation is an underlying cause of many chronic pulmonary diseases such as asthma, chronic obstructive pulmonary disease, and fibrosis [69].

Eicosanoid levels are known to increase in response to inflammatory stimuli in the lungs [70], and PMN infiltration into the pulmonary space is a hallmark feature of pneumococcal pneumonia. While PMN activity is imperative to innate immunity, uncontrolled inflammation can result in tissue destruction and lung disease. In a model of *Streptococcus pneumoniae* infection, 12-LOXs play a major role in PMN migration to the site of pneumococcal infection. Indeed, inhibition of 12-LOX with baicalein prevented the transepithelial infiltration of PMN cells and reduced pulmonary infiltration. Furthermore, depletion of 12-LOX activity in *Alox15*$^{-/-}$ mice led to reduced bacteremia and increased survival as compared to controls where the pulmonary challenge with *S. pneumoniae* was lethal [71]. In an acute lung injury (ALI) mouse model, mouse inhalation of LPS led to induction of inflammation, increased vascular permeability, and upregulation of 12-LOXs. This study also revealed that depletion of 12-LOX lead to significantly reduced vascular permeability upon LPS treatment along with improved gas exchange and increased survival compared to the control littermates [72]. The same group further demonstrated that inflammation in this ALI model is mediated in part via recruitment of neutrophils, as depletion of 12-LOX significantly reduced neutrophil infiltration and prevented edema formation [73]. Therefore, these data demonstrate that 12-LOXs inflammatory activity is crucial in pulmonary infection pathology.

Non-epithelial lung cells appear to exhibit 12-LOX activity in relation to allergic airway inflammation [74][75]. A study showed that intranasal administration of 12-LOX to healthy Balb/c mice leads to airway epithelial injury that promotes airway hyper-responsiveness as seen in asthma. Production of 12-LOX in alveolar macrophages and fibroblasts leads to bronchial epithelial injury via 12-HETE in an IL-13 dependent mechanism [76]. In an allergen-induced lung inflammation model, depletion of 12-LOX (*Alox15*$^{-/-}$) reduced airway inflammation as seen by reduced bronchoalveolar lavage fluid leukocytes (eosinophils, lymphocytes, and macrophages), decreased cytokines (IL-4, IL-5, and IL-13), and reduced luminal mucus secretions in *Alox15*$^{-/-}$ mice compared to wt controls [77]. Recently, it was also shown that deficiency of *Alox15* impairs the granulopoiesis of neutrophils and prevents inflammatory responses to fungal *Aspergillus fumigatus* infection in the lungs [78]. In humans, a hypomethylation of *ALOX12* is associated with asthma in children [79]. Therefore, 12-LOXs participate in lung disease first by affecting infectious inflammation through PMN recruitment and second through promoting increased leukocyte cytokine production in airway hyper-responsiveness.

With respect to human disease, 12-LOXs and 12-HETE have been implicated in inflammatory lung disorders. Lungs of patients exposed to sulfur mustard toxin showed obstructive and restrictive lung disease and an increase in 12-LOX expression compared to control patients [80]. Furthermore, in tuberculosis, the expression of *ALOX12* increases, and it is positively correlated with neutrophil count and bacterial load in the airway [81]. Together these results implicate the 12-LOX pathway in airway epithelial injury relevant to human disease.

### References


1. Cerf, M.E. Beta cell dysfunction and insulin resistance. Front. Endocrinol. 2013, 4, 37.
2. Shoelson, S.E.; Herrero, L.; Naaz, A. Obesity, inflammation, and insulin resistance. Gastroenterology 2007, 132, 2169–2180.
3. Chen, M.; Yang, Z.D.; Smith, K.M.; Carter, J.D.; Nadler, J.L. Activation of 12-Lipoxygenase in proinflammatory cytokine-mediated beta cell toxicity. Diabetologia 2005, 48, 486–495.

4. Wen, Y.; Gu, J.; Chakrabarti, S.K.; Aylor, K.; Marshall, J.; Takahashi, Y.; Yoshimoto, T.; Nadler, J.L. The role of 12/15-Lipoxygenase in the expression of Interleukin-6 and tumor necrosis factor-alpha in macrophages. Endocrinology 2007, 148, 1313–1322.
5. Natarajan, R.; Gerrity, R.G.; Gu, J.L.; Lanting, L.; Thomas, L.; Nadler, J.L. Role of 12-Lipoxygenase and oxidant stress in hyperglycaemia-induced acceleration of atherosclerosis in a diabetic pig model. Diabetologia 2002, 45, 125–133.
6. Miller, Y.I.; Chang, M.K.; Funk, C.D.; Feramisco, J.R.; Witztum, J.L. 12/15-Lipoxygenase translocation enhances site-specific actin polymerization in macrophages phagocytosing apoptotic cells. J. Biol. Chem. 2001, 276, 19431–19439.
7. Zhao, L.; Cuff, C.A.; Moss, E.; Wille, U.; Cyrus, T.; Klein, E.A.; Praticò, D.; Rader, D.J.; Hunter, C.A.; Puré, E.; et al. Selective Interleukin-12 synthesis defect in 12/15-Lipoxygenase-deficient macrophages associated with reduced atherosclerosis in a mouse model of familial hypercholesterolemia. J. Biol. Chem. 2002, 277, 35350–35356.
8. Dobrian, A.D.; Lieb, D.C.; Ma, Q.; Lindsay, J.W.; Cole, B.K.; Kaiwen, M.; Chakrabarti, S.K.; Kuhn, N.S.; Wohlgemuth, S.D.; Fontana, M.; et al. Differential expression and localization of 12/15 Lipoxygenases in adipose tissue in human obese subjects. Biochem. Biophys. Res. Commun. 2010, 403, 485–490.
9. Sears, D.D.; Miles, P.D.; Chapman, J.; Ofrecio, J.M.; Almazan, F.; Thapar, D.; Miller, Y.I. 12/15-Lipoxygenase is required for the early onset of high fat diet-induced adipose tissue inflammation and insulin resistance in mice. PLoS ONE 2009, 4, e7250.
10. Nunemaker, C.S.; Chen, M.; Pei, H.; Kimble, S.D.; Keller, S.R.; Carter, J.D.; Yang, Z.; Smith, K.M.; Wu, R.; Bevard, M.H.; et al. 12-Lipoxygenase-knockout mice are resistant to inflammatory effects of obesity induced by western diet. Am. J. Physiol. Endocrinol. Metab. 2008, 295, E1065–E1075.
11. Cole, B.K.; Morris, M.A.; Grzesik, W.J.; Leone, K.A.; Nadler, J.L. Adipose tissue-specific deletion of 12/15-Lipoxygenase protects mice from the consequences of a high-fat diet. Mediators Inflamm. 2012, 2012.
12. Lee, K.Y.; Russell, S.J.; Ussar, S.; Boucher, J.; Vernochet, C.; Mori, M.A.; Smyth, G.; Rourk, M.; Cederquist, C.; Rosen, E.D.; et al. Lessons on conditional gene targeting in mouse adipose tissue. Diabetes 2013, 62, 864–874.
13. Kinder, M.; Thompson, J.E.; Wei, C.; Shelat, S.G.; Blair, I.A.; Carroll, M.; Puré, E. Interferon regulatory factor-8−driven myeloid differentiation is regulated by 12/15-Lipoxygenase−mediated redox signaling. Exp. Hematol. 2010, 38, 1036–1046.
14. Tersey, S.A.; Maier, B.; Nishiki, Y.; Maganti, A.V.; Nadler, J.L.; Mirmira, R.G. 12-Lipoxygenase promotes obesity-induced oxidative stress in pancreatic islets. Mol. Cell. Biol. 2014, 34, 3735–3745.
15. Tsuru, H.; Osaka, M.; Hiraoka, Y.; Yoshida, M. HFD-induced hepatic lipid accumulation and inflammation are decreased in factor D deficient mouse. Sci. Rep. 2020, 10, 17593.
16. Lazic, M.; Inzaugarat, M.E.; Povero, D.; Zhao, I.C.; Chen, M.; Nalbandian, M.; Miller, Y.I.; Cherñavsky, A.C.; Feldstein, A.E.; Sears, D.D. Reduced dietary Omega-6 to Omega-3 fatty acid ratio and 12/15-Lipoxygenase deficiency are protective against chronic high fat diet-induced steatohepatitis. PLoS ONE 2014, 9, e107658.
17. Machado, M.V.; Michelotti, G.A.; Xie, G.; de Almeida, T.P.; Boursier, J.; Bohnic, B.; Guy, C.D.; Diehl, A.M. Mouse models of diet-induced nonalcoholic steatohepatitis reproduce the heterogeneity of the human disease. PLoS ONE 2015, 10, e0127991.
18. Mori, Y.; Kawakami, Y.; Kanzaki, K.; Otsuki, A.; Kimura, Y.; Kanji, H.; Tanaka, R.; Tsukayama, I.; Hojo, N.; Suzuki-Yamamoto, T.; et al. Arachidonate 12S-Lipoxygenase of platelet-type in hepatic stellate cells of methionine and choline-deficient diet-fed mice. J. Biochem. 2020, 168, 455–463.
19. Tanaka, N.; Matsubara, T.; Krausz, K.W.; Patterson, A.D.; Gonzalez, F.J. Disruption of phospholipid and bile acid homeostasis in mice with nonalcoholic steatohepatitis. Hepatology 2012, 56, 118–129.
20. Rampes, S.; Ma, D. Hepatic ischemia-reperfusion injury in liver transplant setting: Mechanisms and protective strategies. J. Biomed. Res. 2019, 33, 221–234.
21. Zhang, X.-J.; Cheng, X.; Yan, Z.-Z.; Fang, J.; Wang, X.; Wang, W.; Liu, Z.-Y.; Shen, L.-J.; Zhang, P.; Wang, P.-X.; et al. An ALOX12-12-HETE-GPR31 signaling axis is a key mediator of hepatic ischemia-reperfusion injury. Nat. Med. 2018, 24, 73–83.
22. Luci, D.; J. Brian Jameson, I.I.; Yasgar, A.; Diaz, G.; Joshi, N.; Kantz, A.; Markham, K.; Perry, S.; Kuhn, N.; Yeung, J.; et al. Discovery of ML355, a Potent and Selective Inhibitor of Human 12-Lipoxygenase; National Center for Biotechnology Information: Bethesda, MD, USA, 2014.
23. Yang, F.; Zhang, Y.; Ren, H.; Wang, J.; Shang, L.; Liu, Y.; Zhu, W.; Shi, X. Ischemia reperfusion injury promotes recurrence of hepatocellular carcinoma in fatty liver via ALOX12-12HETE-GPR31 signaling axis. J. Exp. Clin. Cancer Res. CR 2019, 38.

24. Subramaniyan, V.; Chakravarthi, S.; Jegasothy, R.; Seng, W.Y.; Fuloria, N.K.; Fuloria, S.; Hazarika, I.; Das, A. Alcohol-associated liver disease: A review on its pathophysiology, diagnosis and drug therapy. Toxicol. Rep. 2021, 8, 376–385.

25. Zhang, W.; Zhong, W.; Sun, Q.; Sun, X.; Zhou, Z. Hepatic overproduction of 13-HODE due to ALOX15 upregulation contributes to alcohol-induced liver injury in mice. Sci. Rep. 2017, 7, 8976.

26. Mumy, K.L.; McCormick, B.A. Events at the host-microbial interface of the gastrointestinal tract II. Role of the intestinal epithelium in pathogen-induced inflammation. Am. J. Physiol. Gastrointest. Liver Physiol. 2005, 288, G854–G859.

27. Sharon, P.; Stenson, W.F. Enhanced synthesis of leukotriene B4 by colonic mucosa in inflammatory bowel disease. Gastroenterology 1984, 86, 453–460.

28. Collins, J.F.; Hu, Z.; Ranganathan, P.N.; Feng, D.; Garrick, L.M.; Garrick, M.D.; Browne, R.W. Induction of arachidonate 12-Lipoxygenase (Alox15) in intestine of iron-deficient rats correlates with the production of biologically active lipid mediators. Am. J. Physiol. Gastrointest. Liver Physiol. 2008, 294, G948–G962.

29. Shannon, V.R.; Stenson, W.F.; Holtzman, M.J. Induction of epithelial arachidonate 12-Lipoxygenase at active sites of inflammatory bowel disease. Am. J. Physiol. Gastrointest. Liver Physiol. 1993, 264, G104–G111.

30. McCormick, B.A.; Colgan, S.P.; Delp-Archer, C.; Miller, S.I.; Madara, J.L. Salmonella typhimurium attachment to human intestinal epithelial monolayers: Transcellular signalling to subepithelial neutrophils. J. Cell Biol. 1993, 123, 895–907.

31. Mrsny, R.J.; Gewirtz, A.T.; Siccardi, D.; Savidge, T.; Hurley, B.P.; Madara, J.L.; McCormick, B.A. Identification of hepoxilin A3 in inflammatory events: A required role in neutrophil migration across intestinal epithelia. Proc. Natl. Acad. Sci. USA 2004, 101, 7421–7426.

32. Kroschwald, S.; Chiu, C.-Y.; Heydeck, D.; Rohwer, N.; Gehring, T.; Seifert, U.; Lux, A.; Rothe, M.; Weylandt, K.-H.; Kuhn, H. Female mice carrying a defective Alox15 gene are protected from experimental colitis via sustained maintenance of the intestinal epithelial barrier function. Biochim. Biophys. Acta BBA Mol. Cell Biol. Lipids 2018, 1863, 866–880.

33. Gibbons, G.H.; Dzau, V.J. The emerging concept of vascular remodeling. N. Engl. J. Med. 1994, 330, 1431–1438. Available online: (accessed on 12 February 2021).

34. Kuhn, H.; Chaitidis, P.; Roffeis, J.; Walther, M. Arachidonic acid metabolites in the cardiovascular system: The role of lipoxygenase isoforms in atherogenesis with particular emphasis on vascular remodeling. J. Cardiovasc. Pharmacol. 2007, 50, 609–620.

35. Setty, B.N.; Graeber, J.E.; Stuart, M.J. The mitogenic effect of 15- and 12-Hydroxyeicosatetraenoic acid on endothelial cells may be mediated via diacylglycerol kinase inhibition. J. Biol. Chem. 1987, 262, 17613–17622.

36. Chava, K.R.; Karpurapu, M.; Wang, D.; Bhanoori, M.; Kundumani-Sridharan, V.; Zhang, Q.; Ichiki, T.; Glasgow, W.C.; Rao, G.N. CREB-mediated IL-6 expression is required for 15(s)-hydroxyeicosatetraenoic acid–induced vascular smooth muscle cell migration. Arterioscler. Thromb. Vasc. Biol. 2009, 29, 809–815.

37. Ross, R. Atherosclerosis–An inflammatory disease. N. Engl. J. Med. 1999, 340, 115–126.

38. Ylä-Herttuala, S.; Rosenfeld, M.E.; Parthasarathy, S.; Glass, C.K.; Sigal, E.; Witztum, J.L.; Steinberg, D. Colocalization of 15-lipoxygenase MRNA and protein with epitopes of oxidized low density lipoprotein in macrophage-rich areas of atherosclerotic lesions. Proc. Natl. Acad. Sci. USA 1990, 87, 6959–6963.

39. Kühn, H.; Heydeck, D.; Hugou, I.; Gniwotta, C. In vivo action of 15-Lipoxygenase in early stages of human atherogenesis. J. Clin. Investig. 1997, 99, 888–893.

40. Kim, P.M.; Kim, J.A.; Harper, C.M.; Shih, P.T.; Berliner, J.A.; Natarajan, R.; Nadler, J.L.; Hedrick, C.C. Lipoxygenase products increase monocyte adhesion to human aortic endothelial cells. Arterioscler. Thromb. Vasc. Biol. 1999, 19, 2615–2622.

41. Kundumani-Sridharan, V.; Dyukova, E.; Hansen, D.E.; Rao, G.N. 12/15-Lipoxygenase mediates high-fat diet-induced endothelial tight junction disruption and monocyte transmigration: A new role for 15(S)-Hydroxyeicosatetraenoic acid in endothelial cell dysfunction. J. Biol. Chem. 2013, 288, 15830–15842.

42. Ylä-Herttuala, S.; Rosenfeld, M.E.; Parthasarathy, S.; Sigal, E.; Särkioja, T.; Witztum, J.L.; Steinberg, D. Gene expression in macrophage-rich human atherosclerotic lesions. 15-Lipoxygenase and acetyl low density lipoprotein receptor messenger RNA colocalize with oxidation specific lipid-protein adducts. J. Clin. Investig. 1991, 87, 1146–1152.

43. Singh, N.K.; Rao, G.N. Emerging role of 12/15-Lipoxygenase (ALOX15) in human pathologies. Prog. Lipid Res. 2019, 73, 28–45.

44. Goerge, J.; Afek, A.; Shaish, A.; Levkovitz, H.; Bloom, B.; Cyrus, T.; Zhao, L.; Funk, C.D.; Sigal, E.; Harats, D. 12/15-Lipoxygenase gene disruption attenuates atherogenesis in LDL receptor–deficient mice. Circulation 2001, 104, 1646–1650.

45. Huo, Y.; Zhao, L.; Hyman, M.C.; Shashkin, P.; Harry, B.L.; Burcin, T.; Forlow, S.B.; Stark, M.A.; Smith, D.F.; Clarke, S.; et al. Critical role of macrophage 12/15-lipoxygenase for atherosclerosis in apolipoprotein E–deficient mice. Circulation 2004, 110, 2024–2031.

46. Cyrus, T.; Pratico, D.; Zhao, L.; Witztum, J.L.; Rader, D.J.; Rokach, J.; FitzGerald, G.A.; Funk, C.D. Absence of 12/15-lipoxygenase expression decreases lipid peroxidation and atherogenesis in apolipoprotein E–deficient mice. Circulation 2001, 103, 2277–2282.

47. Lo Sasso, G.; Schlage, W.K.; Boué, S.; Veljkovic, E.; Peitsch, M.C.; Hoeng, J. The apoe−/− mouse model: A suitable model to study cardiovascular and respiratory diseases in the context of cigarette smoke exposure and harm reduction. J. Transl. Med. 2016, 14.

48. Rong, S.; Cao, Q.; Liu, M.; Seo, J.; Jia, L.; Boudyguina, E.; Gebre, A.K.; Colvin, P.L.; Smith, T.L.; Murphy, R.C.; et al. Macrophage 12/15 lipoxygenase expression increases plasma and hepatic lipid levels and exacerbates atherosclerosis. J. Lipid Res. 2012, 53, 686–695.

49. Heneka, M.T.; Carson, M.J.; El Khoury, J.; Landreth, G.E.; Brosseron, F.; Feinstein, D.L.; Jacobs, A.H.; Wyss-Coray, T.; Vitorica, J.; Ransohoff, R.M.; et al. Neuroinflammation in Alzheimer’s disease. Lancet Neurol. 2015, 14, 388–405.

50. Hirsch, E.C.; Vyas, S.; Hunot, S. Neuroinflammation in Parkinson’s disease. Parkinsonism Relat. Disord. 2012, 18, S210–S212.

51. Jayaraj, R.L.; Azimullah, S.; Beiram, R.; Jalal, F.Y.; Rosenberg, G.A. Neuroinflammation: Friend and foe for ischemic stroke. J. Neuroinflammation 2019, 16, 142.

52. Praticò, D.; Zhukareva, V.; Yao, Y.; Uryu, K.; Funk, C.D.; Lawson, J.A.; Trojanowski, J.Q.; Lee, V.M.-Y. 12/15-Lipoxygenase is increased in Alzheimer’s disease. Am. J. Pathol. 2004, 164, 1655–1662.

53. Czapski, G.A.; Czubowicz, K.; Strosznajder, J.B.; Strosznajder, R.P. The lipoxygenases: Their regulation and implication in Alzheimer’s disease. Neurochem. Res. 2016, 41, 243–257.

54. Kihiko, M.E.; Tucker, H.M.; Rydel, R.E.; Estus, S. C-Jun contributes to amyloid β-induced neuronal apoptosis but is not necessary for amyloid β-induced c-Jun induction. J. Neurochem. 1999, 73, 2609–2612.

55. Lebeau, A.; Terro, F.; Rostene, W.; Pelaprat, D. Blockade of 12-Lipoxygenase expression protects cortical neurons from apoptosis induced by beta-amyloid peptide. Cell Death Differ. 2004, 11, 875–884.

56. Yao, Y.; Clark, C.M.; Trojanowski, J.Q.; Lee, V.M.-Y.; Praticò, D. Elevation of 12/15 lipoxygenase products in AD and mild cognitive impairment. Ann. Neurol. 2005, 58, 623–626.

57. Yang, H.; Zhuo, J.M.; Chu, J.; Chinnici, C.; Pratico, D. Amelioration of the Alzheimer’s disease phenotype by absence of 12/15-Lipoxygenase. Biol. Psychiatry 2010, 68, 922–929.

58. Schapira, A.H.; Agid, Y.; Barone, P.; Jenner, P.; Lemke, M.R.; Poewe, W.; Rascol, O.; Reichmann, H.; Tolosa, E. Perspectives on recent advances in the understanding and treatment of Parkinson’s disease. Eur. J. Neurol. 2009, 16, 1090–1099.

59. Schapira, A.H.; Jenner, P. Etiology and pathogenesis of Parkinson’s disease. Mov. Disord. 2011, 26, 1049–1055.

60. Perry, T.L.; Godin, D.V.; Hansen, S. Parkinson’s disease: A disorder due to nigral glutathione deficiency? Neurosci. Lett. 1982, 33, 305–310.

61. Canals, S.; Casarejos, M.J.; de Bernardo, S.; Rodríguez-Martín, E.; Mena, M.A. Glutathione depletion switches nitric oxide neurotrophic effects to cell death in midbrain cultures: Implications for Parkinson’s disease. J. Neurochem. 2001, 79, 1183–1195.

62. Canals, S.; Casarejos, M.J.; de Bernardo, S.; Rodríguez-Martín, E.; Mena, M.A. Nitric oxide triggers the toxicity due to glutathione depletion in midbrain cultures through 12-Lipoxygenase. J. Biol. Chem. 2003, 278, 21542–21549.

63. Li, Y.; Maher, P.; Schubert, D. A role for 12-Lipoxygenase in nerve cell death caused by glutathione depletion. Neuron 1997, 19, 453–463.

64. van Leyen, K.; Kim, H.Y.; Lee, S.-R.; Jin, G.; Arai, K.; Lo, E.H. Baicalein and 12/15-Lipoxygenase in the ischemic brain. Stroke 2006, 37, 3014–3018.

65. Yigitkanli, K.; Pekcec, A.; Karatas, H.; Pallast, S.; Mandeville, E.; Joshi, N.; Smirnova, N.; Gazaryan, I.; Ratan, R.R.; Witztum, J.L.; et al. Inhibition of 12/15-Lipoxygenase as therapeutic strategy to treat stroke. Ann. Neurol. 2013, 73, 129–135.

66. Moskowitz, M.A.; Kiwak, K.J.; Hekimian, K.; Levine, L. Synthesis of compounds with properties of leukotrienes C4 and D4 in gerbil brains after ischemia and reperfusion. Science 1984, 224, 886–889.

67. Gaberel, T.; Gakuba, C.; Zheng, Y.; Lépine, M.; Lo, E.H.; van Leyen, K. Impact of 12/15-Lipoxygenase on brain injury after subarachnoid hemorrhage. Stroke 2019, 50, 520–523.

68. Seet, R.C.S.; Lee, C.Y.J.; Chan, B.P.L.; Sharma, V.K.; Teoh, H.L.; Venketasubramanian, N.; Lim, E.C.H.; Chong, W.L.; Looi, W.F.; Huang, S.H.; et al. Oxidative damage in ischemic stroke revealed using multiple biomarkers. Stroke 2011, 42, 2326–2329.
69. Huffnagle, G.B.; Dickson, R.P.; Lukacs, N.W. The respiratory tract microbiome and lung inflammation: A two-way street. Mucosal Immunol. 2017, 10, 299–306.
70. Khanapure, S.P.; Garvey, D.S.; Janero, D.R.; Letts, L.G. Eicosanoids in inflammation: Biosynthesis, pharmacology, and therapeutic frontiers. Curr. Top. Med. Chem. 2007, 7, 311–340.
71. Bhowmick, R.; Maung, N.; Hurley, B.P.; Ghanem, E.B.; Gronert, K.; McCormick, B.A.; Leong, J.M. Systemic disease during streptococcus pneumoniae acute lung infection requires 12-Lipoxygenase-dependent inflammation. J. Immunol. 2013, 191.
72. Zarbock, A.; Distasi, M.R.; Smith, E.; Sanders, J.M.; Kronke, G.; Harry, B.L.; von Vietinghoff, S.; Buscher, K.; Nadler, J.L.; Ley, K. Improved survival and reduced vascular permeability by eliminating or blocking 12/15-Lipoxygenase in mouse models of acute lung injury (ALI). J. Immunol. 2009, 183, 4715–4722.
73. Rossaint, J.; Nadler, J.L.; Ley, K.; Zarbock, A. Eliminating or blocking 12/15-Lipoxygenase reduces neutrophil recruitment in mouse models of acute lung injury. Crit. Care 2012, 16, R166.
74. Hajek, A.R.; Lindley, A.R.; Favoreto, S.; Carter, R.; Schleimer, R.P.; Kuperman, D.A. 12/15-Lipoxygenase deficiency protects mice from allergic airways inflammation and increases secretory IgA levels. J. Allergy Clin. Immunol. 2008, 122, 633–639.
75. Lindley, A.R.; Crapster-Pregont, M.; Liu, Y.; Kuperman, D.A. 12/15-Lipoxygenase is an Interleukin-13 and Interferon-γ counterregulated-mediator of allergic airway inflammation. Mediat. Inflamm. 2010, 2010.
76. Mabalirajan, U.; Rehman, R.; Ahmad, T.; Kumar, S.; Leishangthem, G.D.; Singh, S.; Dinda, A.K.; Biswal, S.; Agrawal, A.; Ghosh, B. 12/15-Lipoxygenase expressed in non-epithelial cells causes airway epithelial injury in asthma. Sci. Rep. 2013, 3, 1540.
77. Andersson, C.K.; Claesson, H.-E.; Rydell-Törmänen, K.; Swedmark, S.; Hällgren, A.; Erjefält, J.S. Mice lacking 12/15-Lipoxygenase have attenuated airway allergic inflammation and remodeling. Am. J. Respir. Cell Mol. Biol. 2008, 39, 648–656.
78. Mackel, J.J.; Garth, J.M.; Blackburn, J.P.; Jones, M.; Steele, C. 12/15-Lipoxygenase deficiency impairs neutrophil granulopoiesis and lung proinflammatory responses to aspergillus fumigatus. J. Immunol. 2020.
79. Morales, E.; Bustamante, M.; Vilahur, N.; Escaramis, G.; Montfort, M.; de Cid, R.; Garcia-Esteban, R.; Torrent, M.; Estivill, X.; Grimalt, J.O.; et al. DNA hypomethylation at ALOX12 is associated with persistent wheezing in childhood. Am. J. Respir. Crit. Care Med. 2012, 185, 937–943.
80. Tahmasbpour, E.; Ghanei, M.; Khor, A.; Panahi, Y. Altered expression of Cyclooxygenase-2, 12-Lipoxygenase, inducible nitric oxide synthase-2 and surfactant protein D in lungs of patients with pulmonary injury caused by sulfur mustard. Drug Chem. Toxicol. 2019, 42, 257–263.
81. Mishra, B.B.; Lovewell, R.R.; Olive, A.J.; Zhang, G.; Wang, W.; Eugenin, E.; Smith, C.M.; Phuah, J.Y.; Long, J.E.; Dubuke, M.L.; et al. Nitric oxide prevents a pathogen-permissive granulocytic inflammation during tuberculosis. Nat. Microbiol. 2017, 2, 1–11.