

Platelet-Activating Factor Promotes the Development of Non-Alcoholic Fatty Liver Disease

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Abstract: Non-alcoholic fatty liver disease (NAFLD) is a multifaceted clinicopathological syndrome characterised by excessive hepatic lipid accumulation that causes steatosis, excluding alcoholic factors. Platelet-activating factor (PAF), a biologically active lipid transmitter, induces platelet activation upon binding to the PAF receptor. Recent studies have found that PAF is associated with gamma-glutamyl transferase, which is an indicator of liver disease. Moreover, PAF can stimulate hepatic lipid synthesis and cause hypertriglyceridaemia. Furthermore, the knockdown of the PAF receptor gene in the animal models of NAFLD helped reduce the inflammatory response, improve glucose homeostasis and delay the development of NAFLD. These findings suggest that PAF is associated with NAFLD development. According to reports, patients with NAFLD or animal models have marked platelet activation abnormalities, mainly manifested as enhanced platelet adhesion and aggregation and altered blood rheology. Pharmacological interventions were accompanied by remission of abnormal platelet activation and significant improvement in liver function and lipids in the animal model of NAFLD. These confirm that platelet activation may accompany a critical importance in NAFLD development and progression. However, how PAFs are involved in the NAFLD signalling pathway needs further investigation. In this paper, we review the relevant literature in recent years and discuss the role played by PAF in NAFLD development. It is important to elucidate the pathogenesis of NAFLD and to find effective interventions for treatment.

Keywords: non-alcoholic fatty liver disease, platelet-activating factor, oxidative stress, inflammation, insulin resistance

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a clinicopathological syndrome characterised by excessive fat deposition in hepatocytes, leading to steatosis, excluding alcohol and other definite liver injury factors. NAFLD is a continuous disease spectrum from simple hepatic steatosis (hereafter, NAFL) to non-alcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis and even hepatocellular carcinoma (HCC).¹ Of these, NAFL has a good prognosis, whereas NASH has a significant fibrotic potential and develops frequently. Approximately 32% of the 53% of patients with NASH are progressing to hepatic fibrosis.^{2–8} Even 10–25% of patients with NASH may progress to advanced fibrosis or cirrhosis.^{9–13} In the context of cirrhosis, the incidence of HCC ranges 2–5%.^{14,15} As an important risk factor for liver cancer (HCC), the incidence of HCC associated with advanced fibrosis is reported to be between 2.4% and 12.8%.¹⁶

With the change in people's lifestyles and eating habits, the prevalence rate of NAFLD is increasing annually. Globally, epidemiological surveys have shown that approximately 25% of the population suffers from NAFLD, of which 10–30% is attributed to NASH.¹⁷ The prevalence of NAFLD varies geographically, ie the prevalence is higher in the Middle East and South America, at 32% and 31%, respectively, about 24% in Europe and 14% in Africa. In the United States, the prevalence rate of NAFLD is about 32% and increases with age. In Asia, the overall prevalence rate of NAFLD is about 30%, the onset age is getting younger and the number of patients is increasing annually. Among them, Japan has the lowest, with about 22%. South Korea, Singapore, Malaysia, India and Iran all have prevalence rates of more than 30%, and the prevalence rate in Mainland China is close to 30%^{18–21} (Figure 1). NAFLD has become the number one chronic liver disease worldwide.²²

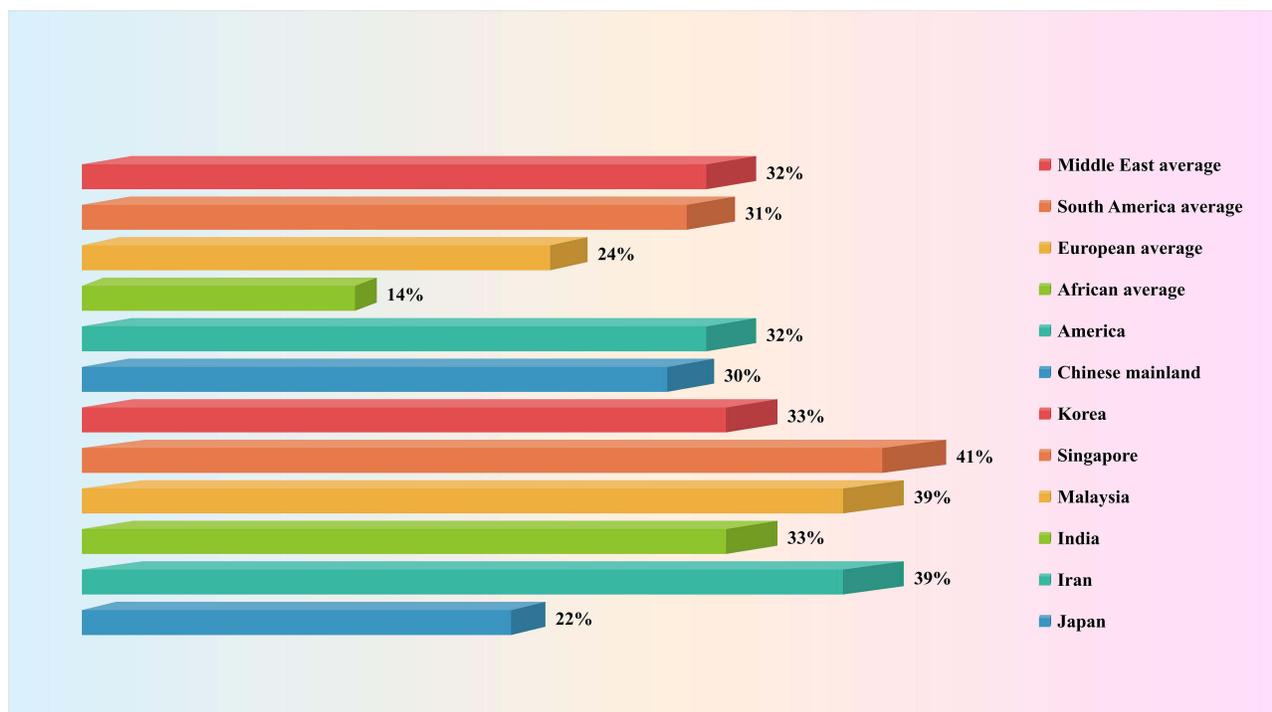


Figure 1 Prevalence of non-alcoholic fatty liver disease in different regions.

The pathogenesis of NAFLD is very complicated. Some risk factors, including lipid metabolism disorder, chronic inflammation and oxidative stress, have been proved to play a key role in the pathogenesis of NAFLD. Currently, the most important pathogenesis of NAFLD is the ‘multiple-hit hypothesis’; the “first hit” is mainly insulin resistance (IR), which inhibits lipid β -oxidation, reduces fatty acid consumption and promotes hepatic triglyceride accumulation. Fat deposition enhances glucose-6-phosphatase activity, stimulates gluconeogenesis and insulin secretion and induces IR,²⁴ leading to a vicious circle of IR and fat deposition. In the first attack, lipid peroxidation produces a large number of reactive oxygen species (ROS), induces oxidative stress, causes liver injury, releases inflammatory cytokines [such as tumour necrosis factor-alpha (TNF- α), interleukin-6 (IL-6) and interleukin-1beta (IL-1 β)] and causes an inflammatory reaction, resulting in the aggravation of liver injury and even necrosis and fibrosis, forming the “second blow”. Moreover, endoplasmic reticulum stress (ERS), mitochondrial dysfunction, adipose tissue dysfunction and changes in intestinal flora can all exacerbate NAFLD development.

To further improve the pathogenesis of NAFLD and complement the “multiple-hit” theory, we propose the hypothesis that the platelet-activating factor (PAF) contributes to NAFLD development. PAF is a phospholipid that can stimulate platelet activation.²⁵ Recent studies have reported the relationship between platelet activation and NAFLD. For example, Oral et al examined plateletcrit in patients with NAFLD and found that it was significantly elevated and positively correlated with the degree of steatosis.²⁶ Similarly, Ozhan et al compared the mean platelet volume (MPV) between patients with NAFLD and patients with non-fatty liver. Their results showed that MPV in patients with NAFLD was higher and positively correlated with IR, a pathogenic factor of NAFLD, and alanine aminotransferase and aspartate aminotransferase.²⁷ Arslan et al also obtained similar results. Through correlation analysis, they confirmed that the increase in MPV in patients with NAFLD was associated with IR.²⁸ Furthermore, Malehmir et al established platelet glycoprotein receptor I $\beta\alpha$ (GPIB α) functionally deficient mice fed a choline-deficient high-fat diet (HFD), which resulted in reduced liver damage, such as hepatic steatosis and inflammatory cell infiltration.²⁹ On the contrary, aspirin, a platelet activation antagonist, has become an important drug for delaying NAFLD development. Clinical aspirin can alleviate NAFLD tissue damage and reduce the prevalence of NAFLD and the risk of liver fibrosis.^{30–33} Consistent with clinical practice, aspirin can also improve NAFLD in animal models. Wang et al used aspirin to treat a mouse model of NAFLD

and significantly improved hepatic steatosis, insulin sensitivity and glucose tolerance.³⁴ Similarly, Ford et al used aspirin to improve blood glucose and insulin tolerance in mice with a HFD. Linear regression analysis confirmed that aspirin could down-regulate proinsulin and improve IR in patients with blood glucose disorders.³⁵ Moreover, Fujita et al used aspirin to reduce steatosis and triglyceride accumulation in the liver of choline-deficient rats and reduce liver inflammatory cell infiltration and fibrosis.³⁶ In line with this, Ibrahim et al administered nitroaspirin to NAFLD rats to attenuate oxidative stress, alleviate hepatic steatosis and reduce inflammatory cytokine-induced expressions of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2).³⁷ This suggests that platelet activation has critical importance in the development and progression of NAFLD. However, how the PAF regulates platelet activation to promote the occurrence and development of NAFLD requires further examination.

To this end, we reviewed a large amount of relevant literature. The results show that the human PAF receptor contains 342 amino acids and belongs to the G protein-coupled receptor family.^{38,39} PAF receptors are widely distributed in tissues and cells, such as platelets, neutrophils, macrophages, monocytes and endothelial cells, in the brain, lungs, liver and uterus.⁴⁰ The binding of the PAF to the PAF receptor induces platelet activation that is involved in NAFLD progression through four main pathways.

The first is the PAF–5-hydroxytryptamine (5-HT) signalling pathway. Most 5-HT in the circulation is stored in platelets, and when the PAF binds to the PAF receptor, it stimulates platelet activation and releases 5-HT. Specific binding of 5-HT to the 5-HT receptor on hepatocytes will activate a series of biological effects. The second is the PAF–inositol triphosphate (IP3) signalling pathway. The PAF activates the hydrolysis of phosphatidylinositol 4,5-bisphosphate in hepatocyte membranes to produce IP3 when bound to the PAF receptor. IP3 binds to inositol 1,4,5-trisphosphate receptor (IP3R) in the hepatocytes, regulates Ca^{2+} release from the endoplasmic reticulum and may trigger ERS. The third is the PAF–arachidonic acid (AA) signalling pathway. Generally, AA is bound to the cell membrane by an ester bond until PLA2 is activated by calcium signalling, at which time it hydrolyses the ester bond and releases AA. Therefore, the PAF, when bound to the PAF receptor, can activate the release of AA from the cell membranes of various cells, including platelets, hepatocytes and inflammatory cells. AA can be further converted into leukotrienes (LTs), prostaglandins (PG) and thromboxane A2 (TXA2). PG mainly includes signalling molecules, such as prostaglandin I2 (PGI2), prostaglandin E2 (PGE2), prostaglandin D2 (PGD2) and prostaglandin F2 α (PGF2 α). The fourth pathway is the PAF–diacylglycerol (DAG) signalling pathway. The PAF, when combined with the PAF receptor, activates the hydrolysis of phosphatidylinositol 4,5-bisphosphate in the hepatocyte membranes to produce DAG. DAG deposition in hepatocytes may induce the activation of p38-mitogen-activated protein kinases (p38MAPK) and protein kinase C (PKC) (protein kinase C ϵ and protein kinase C δ). Protein kinase C δ can also agonise ERS. These signalling pathways can cause cascading reactions that lead to oxidative stress, inflammatory responses or IR and damage to hepatocytes and even the liver, which may ultimately contribute to NAFLD development and progression (Figure 2).

PAF-Mediated Cytokine Involvement in NAFLD

PAF–5-HT Signalling Pathway and NAFLD

The PAF–5-HT signalling pathway occurs mainly by PAF-induced platelet activation, which stimulates the release of 5-HT from platelet-dense granules. 5-HT is involved in platelet aggregation and other processes by binding to the 5-HT receptor. This phenomenon is supported by clinical data, for example, Greco et al used PAF to treat platelets in patients with type 1 diabetes, resulting in the up-regulation of 5-HT release over time and inducing platelet aggregation.⁴¹ Similar results were obtained in the experiments of O'Donnell et al They incubated PAF with human platelets, which significantly increased the release of 5-HT.⁴² Consistent with clinical data, PAF also has an excitatory effect on 5-HT in animal experiments. This was confirmed in an experiment by Murphy et al, who used PAF to stimulate rabbit platelets and promoted the secretion of 5-HT.⁴³ Similar results were obtained in the experiments by Pédroneo et al The incubation of rabbit platelets with increasing PAF concentrations resulted in a concentration-dependent release of 5-HT.⁴⁴ Moreover, Brooks et al activated horse platelets with PAF to trigger 5-HT release and promote platelet aggregation.⁴⁵ In line with this, similar results were reported in an *in vitro* experiment by Bailey et al who used PAF to treat horse platelets, resulting in 5-HT release and platelet activation products, such as thromboxane-B2 (TXB2) and 12-hydroxyeicosatetraenoic acid

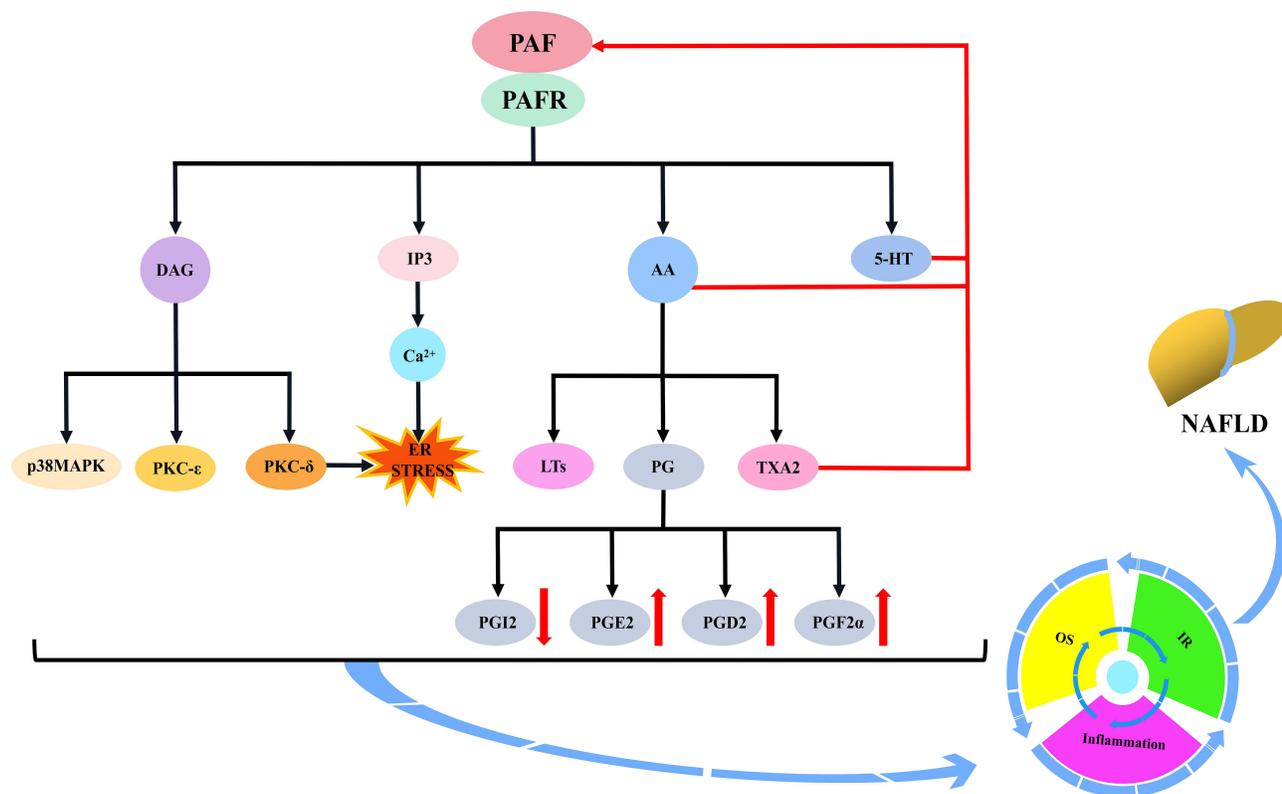


Figure 2 Mechanism of action of the PAF via platelet activation to induce NAFLD. The binding of the PAF to the PAFR will induce platelet activation and stimulate the secretion of 5-HT, IP₃, AA and DAG by platelets or multiple cells. IP₃, by regulating Ca²⁺ mobilisation, may trigger endoplasmic reticulum stress (ERS). AA can be further converted to LTs, PG and TXA₂. PG mainly includes PGI₂, PGE₂, PGD₂ and PGF₂α. The activation of DAG induces the activation of p38MAPK and PKC (PKCε and PKCδ), and PKCδ may agonise ERS. Moreover, 5-HT, AA and TXA₂ further stimulate the PAF and amplify platelet activation. Subsequently, these substances induce cascade reactions that may be involved in NAFLD development by promoting the development of oxidative stress, inflammatory responses or insulin resistance.

Abbreviations: PAF, platelet-activating factor; PAFR, platelet-activating factor receptor; 5-HT, 5-hydroxytryptamine; IP₃, inositol triphosphate; AA, arachidonic acid; DAG, diacylglycerol; ER STRESS, endoplasmic reticulum stress; LTs, leukotrienes; PG, prostaglandins; TXA₂, thromboxane A₂; PGI₂, prostaglandin I₂; PGE₂, prostaglandin E₂; PGD₂, prostaglandin D₂; PGF₂α, prostaglandin F₂α; p38MAPK, p38-mitogen-activated protein kinases; PKC, protein kinase C; PKCε, protein kinase Cε; PKCδ, protein kinase Cδ; OS, oxidative stress; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease.

(12-HETE). Based on this, the PAF–5-HT signalling pathway is formed.⁴⁶ 5-HT is synthesised by tryptophan hydroxylase (TPH) and 5-hydroxytryptophan decarboxylase from tryptophan; it is secreted by intestinal chromaffin cells, widely distributed in the circulation. Most of the 5-HT in the blood circulation will be absorbed and sequestered into platelets. Under PAF stimulation, 5-HT is released from platelets and will enter the liver and other organs for metabolism and participate in the pathological processes of some liver diseases.^{47–49}

5-HT is a well-known modulator of liver functions. During the onset of chronic liver diseases, the elevated plasma levels of 5-HT induce oxidative stress, impair insulin sensitivity and promote the activation of multiple inflammatory signalling molecules in hepatocytes, all of which play a key role in hepatic steatosis, dyslipidaemia and liver injury (Figure 3).^{50–53}

5-HT needs to bind to its specific receptor for executing biological functions. The presence of 5-HTR2A on the surface of hepatocytes suggested that 5-HT release induced by PAF may be involved in the disease processes in the liver, eg NAFLD.⁵⁴ In this regard, Osawa et al used 5-HT to treat hepatocytes and, as a result, significantly increased triglyceride levels and lipid droplet accumulation.⁵⁵ Similarly, Wang et al detected 5-HTR2A expression in liver tissues and hepatocytes (BRL-3A), and they treated hepatocytes with an HTR2A agonist (TCB-2) and an antagonist (ketanserin), respectively. Their results showed that TCB-2 promoted the mRNA expression of peroxisome proliferator-activated receptor γ 2 (*PPAR* γ 2), sterol regulatory element-binding transcription factor 1c (*SREBP-1C*) and fatty acid synthase (*FAS*), genes related to hepatocyte lipid synthesis, and increased hepatocyte neutral lipid and triglyceride levels.⁵⁴ Consistent with cellular experiments, Wang et al also confirmed in animal models that 5-HT is associated with NAFLD, and they used a high-

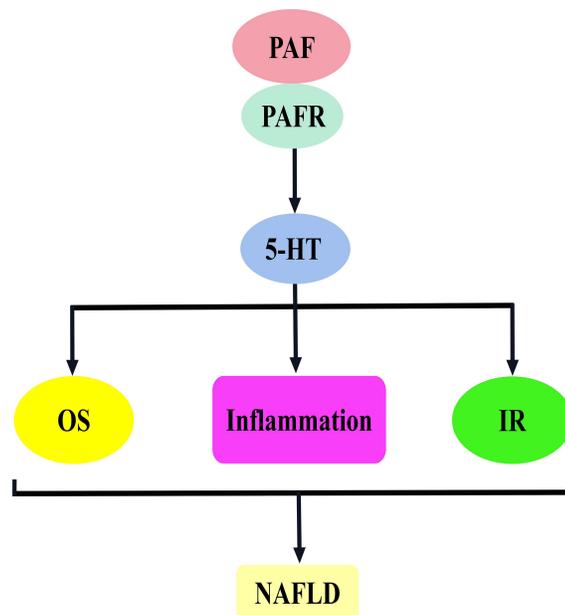


Figure 3 PAF activates 5-hydroxytryptamine to promote the mechanism of NAFLD development. The binding of PAF to PAFR stimulates 5-HT release, which is involved in NAFLD development mainly by inducing oxidative stress, inflammatory response and insulin resistance.

Abbreviations: PAF, platelet-activating factor; PAFR, platelet-activating factor receptor; 5-HT, 5-hydroxytryptamine; OS, oxidative stress; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease.

fat, high-sugar diet to induce a NASH rat model. Their results showed an up-regulation of serum 5-HT levels and a positive correlation with the non-alcoholic fatty liver activity score (NAS).⁵⁴ Similarly, Osawa et al used L-tryptophan (5-HT raw material) in combination with a high-fat chow to feed mice, which resulted in the steatosis of the liver accompanied by elevated serum 5-HT levels.⁵⁵ In addition, clinical data confirm the correlation between 5-HT and NAFLD. For instance, Wang et al compared serum 5-HT levels in patients diagnosed with NAFLD by ultrasonography with those healthy individuals. As shown in the results, the serum 5-HT level was significantly higher in patients with NAFLD than in normal subjects. Moreover, they further analysed and confirmed that serum 5-HT was positively correlated with ultrasound scores. Serum 5-HT may be associated with NAFLD.⁵⁴ However, the mechanism of 5-HT involvement in NAFLD needs further investigation. In this regard, Nam et al used LP-533401 or p-chlorophenyl alanine (a TPH inhibitor) to treat mice on a HFD. As results showed, hepatic steatosis was improved and the expression of lipid metabolism-related genes, such as *Srebp-1c*, *FAS* and apolipoprotein b (*ApoB*) was significantly down-regulated. It was confirmed that 5-HT regulation of lipid metabolism drives NAFLD development.⁵⁶ Similar results were obtained in a trial by Choi et al, who knocked out the tryptophan hydroxylase 1 (*Tph1*) or *Htr2a* genes in mice on a HFD, blocked 5-HT signalling, attenuated hepatic steatosis and reduced the expression of adipogenesis-related genes.⁵⁷ Notably, 5-HT can induce multiple signals downstream to drive NAFLD development. For example, Wang et al used a free fatty acid (FFA)-induced NASH hepatocyte model and treated hepatocytes with an HTR2A agonist (TCB-2) and an antagonist (ketanserin), respectively. Their results showed that TCB-2 significantly up-regulated the mRNA expressions of *Tnf- α* , *Il-6* and monocyte chemoattractant protein 1 (*Mcp-1*) compared with ketanserin.⁵⁴ This indicated that 5-HT-mediated inflammatory responses accelerate NASH progression. Similar results were obtained in a trial by Crane et al, who knocked down the *Tph1* gene in mice on a HFD, resulting in a significant reduction in the adipose tissue inflammatory markers cluster of differentiation 68 (*Cd68*), *Tnf- α* and *Mcp-1*.⁵⁸ In addition, Crane et al further found that *Tph1* knockout mice had lower blood glucose and fasting serum insulin. Moreover, glucose tolerance and insulin sensitivity were improved. This indicated that 5-HT is involved in NAFLD via IR.⁵⁸ Oxidative stress may be another mechanism by which 5-HT is involved in NAFLD. This was verified in a trial by Nocito et al, who used a choline-methionine-deficient diet to induce a mouse model of NASH with significantly elevated liver tissue malondialdehyde (MDA). Interestingly, the knockdown of the *Tph1* gene reversed this effect.⁵⁹

PAF–IP3–Ca²⁺ Signalling Pathway and NAFLD

The activation of the PAF–IP3–Ca²⁺ signalling cascade depends on the intracellular signal transduction of PAF. By activating phospholipase C, phosphatidylinositol 4,5-bisphosphate is hydrolysed to induce IP3 production and further mobilisation of intracellular Ca²⁺ release.⁶⁰ This effect is present in platelets and cells. For example, Sakon et al used PAF to incubate platelets, which increased the concentration of Ca²⁺ by stimulating IP3 formation.⁶¹ Similarly, Yu et al induced human platelets by using PAF, which significantly promoted the IP3 production and elevated Ca²⁺ concentration.⁶² Moreover, the effect of PAF to mobilise Ca²⁺ is present in monocytes. This was confirmed by Ng et al in their experiments; they used PAF to treat human peripheral blood mononuclear cells by inducing phosphatidylinositol hydrolysis, leading to a dose-dependent release of IP3, which triggered Ca²⁺ mobilisation.⁶³ However, L-659, L-989 or WEB2086 (PAF receptor antagonists) abolished this effect.^{63,64} Accordingly, in endothelial cells, Lin et al demonstrated that PAF stimulates phosphatidylinositol metabolism in a dose-dependent manner, generating IP3 and causing Ca²⁺ elevation, an effect inhibited by WEB2086 (PAF receptor antagonist).⁶⁵ In neutrophils, Koike et al showed that PAF treatment increased IP3 levels and mobilised the release of intracellular Ca²⁺ and that SM-12502, WEB-2086 and RP-48740 (PAF receptor antagonists) blocked this effect.⁶⁶ This effect is also present in hepatocytes. Okayasu et al incubated rat hepatocytes with PAF and detected a down-regulation of phosphatidylinositol-4,5-bis-phosphate, suggesting the synthesis of IP3.⁶⁷ However, IP3 needs to bind to IP3R to mobilise Ca²⁺ release. This view is supported by Chu et al; they used PAF pre-treatment of guinea pig-isolated cardiomyocytes, which promoted Ca²⁺ mobilisation through the activation of the IP3 pathway. Interestingly, this effect was eliminated by the IP3R antagonist 2-aminoethoxydiphenyl borate.⁶⁸ This suggested that IP3 downstream signalling requires the help of the IP3R. This conclusion was supported by Lautenschläger et al, who used PAF to stimulate Ca²⁺ release downstream of IP3 and slowed down small intestinal vasoconstriction; in addition, further use of 2-aminoethoxydiphenyl borate to weaken IP3 signalling reversed this effect.⁶⁹ For this reason, the PAF–IP3–Ca²⁺ signalling pathway was formed.⁷⁰ IP3 is a signalling messenger whose main role is to mobilise intracellular Ca²⁺ release. In response to PAF stimulation, IP3 is produced in large quantities. By binding to the IP3R, IP3 activates the Ca²⁺ channel and stimulates Ca²⁺ transport, thereby promoting mitochondrial Ca²⁺ inward flow,⁷¹ which contributes to cellular injury and liver disease development.⁷¹

IP3R is mainly located in the endoplasmic reticulum; moreover, binding to IP3 induces receptor conformational changes and channel opening, which mediates Ca²⁺ release and transfer from the endoplasmic reticulum to mitochondria.⁷² When Ca²⁺ release is uncontrolled, it causes mitochondrial calcium overload and functional impairment. In case of serious uncontrolled release, it induces ERS, oxidative stress, inflammatory response and IR as well as damage to hepatocytes, which can lead to various liver diseases, including NAFLD (Figure 4).^{73,74}

In hepatocytes, the IP3R is the only intracellular calcium release channel. In this regard, Hirata et al used IP3 to stimulate hepatocytes, and as a result, the expression of the IP3R and Ca²⁺ waves was detected.⁷⁵ After induced release by PAF in hepatocytes, IP3 may bind to the IP3R to regulate Ca²⁺ signalling and be involved in the development of liver disease. Therefore, NAFLD progression is usually accompanied by the dysregulation of IP3R expression.⁷⁶ This idea is supported by Arruda et al, who examined mitochondrial proteins in obese mouse hepatocytes. As a result, the expression of proteins related to the Ca²⁺ transport, such as IP3R type 1 (IP3R1), was significantly increased. This causes Ca²⁺ overload, impairs mitochondrial membrane potential and leads to mitochondrial dysfunction. Subsequently, the phosphorylation of insulin receptors insulin receptor substrate 1 (IRS1) and protein kinase B (AKT) are blocked, resulting in impaired insulin sensitivity and glucose tolerance. This contributes to NAFLD development. However, the knockdown of the IP3R type 1 (*Ip3r1*) gene significantly improved the Ca²⁺ flux to avoid these results.⁷⁷ Accordingly, Feriod et al obtained *Ip3r1* gene deletion mice using a hybridisation technique. As a result, hepatic *Ip3r1* protein expression was reduced, resulting in impaired Ca²⁺ signalling; meanwhile, triglycerides were down-regulated, steatosis was improved and expressions of lipogenic genes *Srebp* and *FAS* were reduced.⁷⁸ The suppression of the *Ip3r1* gene has good anti-NAFLD activity. The IP3R opens only when it binds to IP3, inducing the Ca²⁺ release from the endoplasmic reticulum.⁷⁹ In NAFLD, sustained IP3 stimulation will result in a large loss of Ca²⁺ from the endoplasmic reticulum, which will activate the calcium channel store-operated calcium channel (SOCC) that mediates the inward flow of extracellular Ca²⁺^{80–82} leading to an increase in intracellular Ca²⁺ concentration and causing calcium imbalance in the endoplasmic reticulum. This was confirmed in the

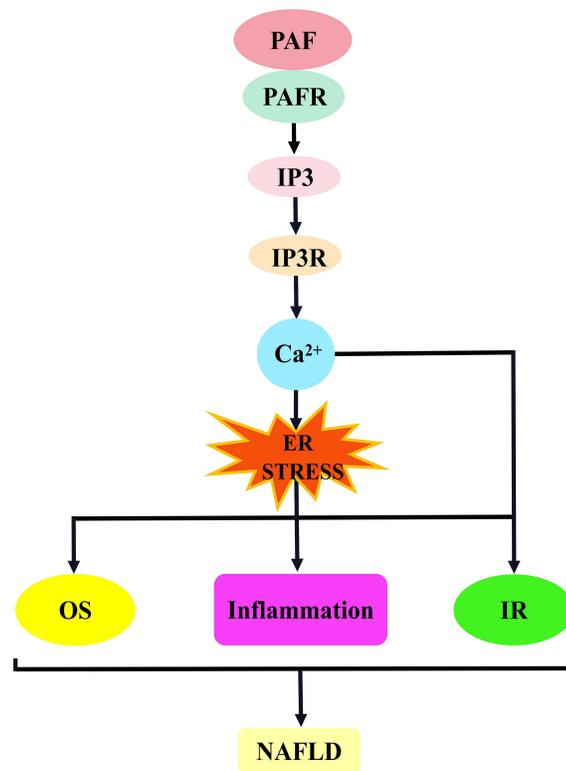


Figure 4 PAF activates inositol triphosphate to promote the mechanism of NAFLD development. The binding of PAF to PAFR induces IP3 production, and IP3 binding to the IP3R promotes Ca^{2+} release, damages mitochondria and induces IR. Moreover, Ca^{2+} overload may induce ER stress. ER stress is associated with the development of oxidative stress, inflammatory responses and insulin resistance, which contribute to NAFLD development.

Abbreviations: PAF, platelet-activating factor; PAFR, platelet-activating factor receptor; IP3, inositol triphosphate; IP3R, inositol 1,4,5-trisphosphate receptors; ER STRESS, endoplasmic reticulum stress; OS, oxidative stress; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease.

experiments by Zhang et al They established a mouse hepatocyte lipid gradient model using oleic acid, and their results showed a concentration-dependent increase in 1,4,5-trisphosphate receptor and SOCC channel opening and intracellular calcium levels with increasing oleic acid concentration.⁸³ Endoplasmic reticulum calcium imbalance predisposes to ERS.⁸⁴ Thus, Lai et al used palmitic acid (PA) in L02 cells to induce a hepatic steatosis cell model. Their results showed that reduced sarco-endoplasmic reticulum Ca^{2+} -ATPase (SERCA) activity led to the up-regulation of intracellular calcium ion concentration and increased the expression of ERS-related proteins binding immunoglobulin protein (BIP), C/EBP homologous protein (CHOP), activating transcription factor-6 and inositol-requiring protein-1 (IRE-1). These prompts that ERS is activated.⁸⁵ Similar results were obtained in the experiments by Zhang et al, who used PA to treat hepatocytes, which impaired SERCA activity and disrupted endoplasmic reticulum Ca^{2+} homeostasis, leading to ERS.⁸⁶ Oxidised protein folding releases a single ROS for each disulphide bond formed, whereas the endoplasmic reticulum provides a unique environment for protein folding and disulphide bond generation. Consequently, ERS generates large amounts of ROS, leading to increased oxidative stress, which contributes to NAFLD development.⁸⁷ Interestingly, ROS is also thought to be one of the main causes of ERS induction, forming a vicious circle with ERS. This was confirmed in an experiment by Zhang et al, who used PA to treat hepatocytes and significantly up-regulated ROS levels, resulting in ERS.⁸⁶ Further inhibition of ROS using the hepatic stimulating substance was accompanied by the down-regulation of ERS-related marker expression. In addition, by enhancing inflammatory or apoptotic signalling, ERS may be another mechanism to promote NAFLD.^{88,89} The experiments by Zhang et al confirmed this idea. They administered the ERS-inducing agent clathrin tunicamycin intraperitoneally to mice, which resulted in hepatic steatosis with significant increases in ERS markers, including BIP/glucose-regulated protein 78 (BIP/GRP78), CHOP and IRE1 α . Meanwhile, the inflammatory response is enhanced, and IL-1 β expression is up-regulated. Further knockdown of the *caspase-1* gene significantly improved these results. It is implied that inflammatory vesicle activity is also involved in ERS-induced hepatic steatosis and

inflammation.⁹⁰ Similarly, Xu et al used ginsenoside Rg1 (Rg1) to inhibit the activation of NOD-like receptor family pyrin domain-containing 3 inflammatory vesicles and IL-1 β and IL-18 by reducing the expressions of ERS-related proteins GRP78 and CHOP in NAFLD mice.⁹¹ Moreover, Ye et al added ERS agonists in a NASH cell model to activate the expressions of nuclear factor kappa B (NF- κ B)-related proteins, and the ERS inhibitor taurine deoxycholic acid reverses this phenomenon.⁹² ERS may also promote NAFLD progression by activating IR. For example, Özcan et al used the ERS inducer clathrin (tunicamycin) to treat hepatocytes. Accordingly, tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1) was decreased, and AKT phosphorylation was inhibited, promoting IR and accelerating NAFLD progression.⁹³ Similarly, Arruda et al utilised a recombinant construct encoding a synthetic linker that increased the endoplasmic reticulum to mitochondrial contact sites and facilitated Ca²⁺ transport from the endoplasmic reticulum to the mitochondria. In the HFD mice, this linker leads to the impaired phosphorylation of IRS1 and AKT in the liver, activated the expression of c-Jun N-terminal kinase (JNK), activating transcription factor-4 (ATF4) and CHOP proteins as well as activating transcription factor-3 (*Atf3*) and *Atf4* mRNA. ERS may be present during IR development.⁷⁷

PAF-AA Signalling Pathway and NAFLD

The activation of the PAF-AA signalling pathway is dependent on PAF-driven phospholipase A2 (PLA2) activation, which regulates AA production.⁹⁴ In platelets and cells, PAF has a strong stimulatory activity against AA. This idea is supported by Catalán et al, as they used PAF to pretreat rabbit platelets. Accordingly, AA sustained a rapid release, and this effect was eliminated by the dihydropyridine derivative PCA-4230, probably because this drug affects a PAF receptor antagonist.⁹⁵ Analogously, similar results were obtained in the experiments by Oestvang et al; they employed PAF to treat THP-1 monocytes. As a result, PLA2 activation was induced, triggering AA release. However, the PAF antagonist WEB2170 abolished the synthesis of AA.⁹⁶ Moreover, Hurst et al used PAF to stimulate rabbit corneas, resulting in the up-regulation of AA release with time. However, the addition of BN50727 (PAF receptor antagonist) effectively attenuated PAF signalling, thus reversing this effect.⁹⁷ The same effect is present in hepatocytes. The incubation of hepatocytes with PAF by Svetlov et al significantly increased the content of free AA.⁹⁸ From this, the PAF-AA signalling pathway is formed. AA is an essential fatty acid, mainly used for the synthesis of pro-inflammatory mediators. They activate intracellular inflammatory signal transduction after binding to inflammatory cell receptors. This contributes to the occurrence of cardiovascular diseases, liver diseases, etc.⁹⁹

AA is a polyunsaturated fatty acid, which is closely related to metabolic disorders. The development of NAFLD, as a chronic metabolic liver disease, necessitates the use of AA.¹⁰⁰ The increase in AA (an inflammatory regulatory mediator) levels contributes to cellular inflammatory signalling. Interestingly, AA deposition is toxic to cells, which disrupts cellular oxidative stress homeostasis and causes cell damage.^{101,102} In addition, AA can generate hydroperoxides catalysed by LOX, which induces oxidative stress, inflammatory response and IR, thereby leading to the worsening of NAFLD (Figure 5).¹⁰³

PAF is a known phospholipid mediator of inflammation and is a potent chemokine for various inflammatory cells. It activates the inflammatory cascade through G protein-coupled receptors, stimulating inflammatory cell production and releasing inflammatory mediators, such as TNF- α , IL-1 and IL-6, thereby increasing vascular permeability and leading to a vascular inflammatory response.^{104,105} Normally, AA is bound to the cell membrane as an ester bond until PLA2 is activated through calcium signalling to hydrolyse the ester bond and release AA. AA will synthesise pro-inflammatory mediators, activate intracellular inflammatory signals, promote the synthesis and release of inflammatory factors, such as TNF and ILs, and amplify the inflammatory response.^{106,107} This suggests that PAF can promote inflammatory responses by activating PLA2, thereby releasing AA. Inflammation is a constant in NAFLD.¹⁰⁸ Elevated levels and activation of inflammatory cytokines underlie the pathology of hepatocyte injury.¹⁰⁹ PAF-AA signalling may drive NAFLD development by damaging hepatocytes. Thus, NAFLD progression is usually accompanied by the dysregulation of the expression of AA, the inflammatory pathway-related protein PLA2. This view is supported by clinical data that confirm this view. Colak et al compared the levels of PLA2, the key enzyme of AA production, in the serum of patients with NASH and healthy participants. Their results showed that PLA2 levels were elevated in patients with NASH and significantly correlated with steatosis scores.¹¹⁰ Moreover, Zelber et al used liquid chromatography-tandem mass spectrometry to determine AA levels in 105 patients with NAFLD. Their results confirmed that AA levels were elevated

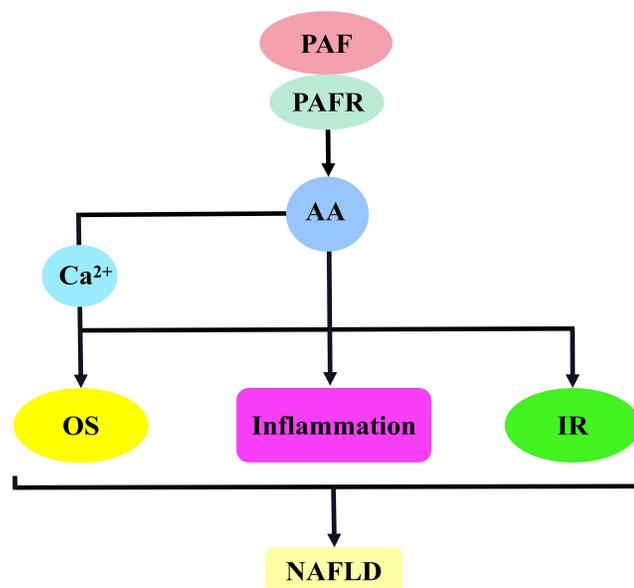


Figure 5 PAF activates AA to promote the mechanism of NAFLD development. The binding of PAF to PAFR stimulates AA release, which is involved in NAFLD development mainly by inducing oxidative stress, inflammatory response and IR.

Abbreviations: PAF, platelet-activating factor; PAFR, platelet-activating factor receptor; AA, arachidonic acid; OS, oxidative stress; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease.

in patients with NAFLD and were not associated with obesity.¹¹¹ Similarly, Tutino et al used aerobic exercise and a hypoglycaemic diet to down-regulate the ratio of pro-inflammatory AA to the anti-inflammatory antioxidant eicosapentaenoic acid (EPA) in patients with NAFLD, resulting in improved steatosis. Furthermore, they developed a linear regression model to confirm that a decrease in the AA/EPA ratio was associated with an improvement in NAFLD.¹¹² Consistent with clinical data, animal experiments have confirmed that with dysregulated PLA2, AA mediates inflammation to promote NAFLD development. Ii et al attenuated HFD induced hepatic steatosis by knocking out the *Pla2* gene in HFD mice.¹¹³ Furthermore, Sztolszterer et al induced an NAFLD rat model with a HFD. Their results showed that the expressions of inflammation-related proteins NF- κ B and IL-6 are elevated with the increase in AA content.¹¹⁴ Similarly, Ma et al used a HFD to establish an NAFLD rat model, and their results showed that *PLA2* gene expression, which promoted AA production, led to the expression of pro-inflammatory mediators and exacerbated hepatic steatosis.¹¹⁵ ROS signalling activated by AA may be another mechanism that promotes NAFLD. This was verified in the experiments by Ghazali et al, who treated HepG2 cells with different ratios of AA/docosahexaenoic acid (DHA), and their results showed that a high AA/DHA ratio could inhibit mitochondrial respiration and activity, causing mitochondrial dysfunction, inducing ROS production and thus exacerbating oxidative stress.¹¹⁶ This contributes to NAFLD development. Interestingly, AA can activate cell membrane Ca²⁺ channels, leading to Ca²⁺ inward flow,^{117,118} which activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and promotes ROS release.¹¹⁹ Conversely, ROS can trigger AA production signals and enhance AA-related metabolism, creating a cyclic effect.¹²⁰ The metabolic pathways of AA may also contribute to NAFLD development. For example, 12/15-lipoxygenase (12-LOX) enhances the IR and inflammatory response to accelerate the NAFLD process. This opinion is supported by Nunemaker et al, who knocked out the 12-Lox gene in mice on a HFD by a reverse experiment, resulting in improved IR and macrophage infiltration within adipocytes.¹²¹ Similarly, Lazic et al obtained similar results. They knocked out the 12-Lox gene from the whole body of mice on a HFD, resulting in reduced steatosis and macrophage infiltration in the liver, decreased expression of pro-inflammatory cytokine genes (interferon- γ (*Ifn- γ*), *Tnf- α* genes and *Il-10* mRNA) and decreased immunocyte chemokine microphage inflammatory protein 2 (*Cxcl2/3*) in the liver.¹²² Moreover, 5-LOX can accelerate NAFLD progression. This was confirmed in the experiments by Martínez et al 5-LOX is activated in APOE-/- mice, mainly through the NF- κ B-induced activation of hepatic pro-inflammatory cytokines (*Tnf- α* , *Mcp-1* and *Il-18*). This would further stimulate macrophage infiltration and *Caspase-3* activation, driving hepatitis development. However, the deletion

of the 5-Lox gene mitigates this effect.¹²³ In addition, AA metabolites have been associated with NAFLD development. Puri et al compared the LOX pathway products of patients with NASH with those of healthy individuals. Metabolomics has shown increased LOX pathway products in patients with NASH, including 5-hydroxyeicosatetraenoic acid (5-HETE) and 15-hydroxyeicosatetraenoic acid (15-HETE).¹²⁴ Similarly, Hall et al used a HFD to establish a NASH mouse model and detected increases in 12-hydroxyeicosatetraenoic acid (12-HETE, 15-HETE and 5-HETE).¹²⁵ Moreover, Ma et al treated human islets with 12-HETE and showed reduced insulin secretion and β -cell dysfunction, suggesting that 12-HETE is associated with islet stress.¹²⁶ Similarly, Chakrabarti et al used 12-HETE or 15-HETE to treat 3T3-L1 adipocytes, and their results show that 12-HETE or 15-HETE triggers the secretion of inflammatory adipokines and impairs the action of insulin, suggesting a contribution to NAFLD development.¹²⁷

PAF-AA-LTs Signalling Pathway and NAFLD

The PAF-AA-LTs signalling cascade is dependent on the activation of the PAF-AA signalling pathway. Then, through the 5-LOX metabolic pathway, it induces the activation of downstream inflammatory factor LTs. This will promote the occurrence of inflammatory reactions.¹²⁸ Thus, AA is highly agonistic for LTs in platelets and multiple cellular tissues. This idea was verified by the experiments by Lecomte et al who incubated human platelets with AA, resulting in the detection of LTC4-like substances.¹²⁹ This effect is also present in hepatocytes. Otomo et al used AA to treat rat hepatocytes and showed a concentration-dependent production of LTs.¹³⁰ AA-induced synthesis of LTs requires the catalysis of 5-LOX. This was confirmed in the experiment by Doskey et al 2,3,7,8-Tetrachlorodibenzo-p-dioxin induced the activation of the 5-LOX pathway via AA, which further stimulated the secretion of leukotriene B4 (LTB4) and leukotriene B3 (LTB3).¹³¹ Similarly, Takasugi et al used AA pre-treatment of mast cells, which triggered the release of LTB4. Interestingly, MK-886 (a LOX inhibitor) abolished this process. This indicated that LTB4 is produced via the 5-LOX pathway.¹³² Moreover, Ito et al used endotoxin lipopolysaccharide (LPS) to induce impaired hepatic micro-circulation to explore the role of LTB4 and LTB4 receptor type 1 (*BLTI*). Consequently, LPS can trigger LTB4 release and cause liver injury by activating the AA-dependent 5-LOX pathway. However, AA-861 (5-LOX synthase inhibitor) or *BLTI* knockdown can block AA or LTs signalling and eliminate this effect.¹³³ Accordingly, the PAF-AA-LTs signalling pathway was formed. LTs are a class of highly biologically active inflammatory mediators. They amplify the inflammatory response by acting on the corresponding receptors on inflammatory cells and chemotactic inflammatory cell infiltration and modulating the intensity and duration of inflammation, thus affecting the liver disease process.¹³⁴

Chronic inflammation is believed to be a key pathophysiological mechanism behind IR. Leukotrienes are potent inflammatory cell chemoattractants that mediate inflammatory responses and promote the development of NAFLD. Moreover, excess leukotrienes induce inflammation-associated IR, reduce insulin sensitivity and exacerbate liver damage (Figure 6).¹³⁵

During NAFLD development, AA plays an important role as a regulator of the inflammatory cascade response.^{34,136} LTs, a downstream product of AA, are usually synthesised under the catalysis of 5-LOX.¹²⁸ In liver and adipose tissue of experimentally obese mice, the expression and activity of enzymes required for LTs biosynthesis, including 5-LOX and 5-LOX-activated protein, were significantly increased.¹³⁷⁻¹³⁹ This suggests that AA induces the release of large amounts of LTs through the catalysis of 5-LOX; subsequently, the AA-LTs signalling cascade activates an inflammatory response that drives NAFLD.^{115,140} This view was confirmed by Ma et al They established a NAFLD rat model with a HFD to explore the role of the AA-5-LOX pathway in the pathogenesis of NAFLD. As a result, AA increased significantly as NAFLD progressed, promoting the release of cysteinyl-LTs (CysLTs) through enhanced 5-LOX protein expression. This contributes to NAFLD development. Moreover, they conducted reverse experiments using cysLTs and a 5-LOX pathway inhibitor (zileuton). The inhibition of the AA-5LOX signalling pathway delays NASH progression.¹¹⁵ Indeed, the AA-LTs signalling pathway leads to stable high levels of inflammatory LTB in NAFLD, which contributes to disease progression.¹³⁶ In this regard, Horrillo et al used reversed-phase high-performance LC to analyse 5-LOX products in obese mice; as a result, the level of the pro-inflammatory 5-LOX product LTB4 was increased.¹³⁸ In line with this, Chakrabarti et al analysed Zucker rat adipocytes using high-performance LC. Their results showed the up-regulation of LTB4 expression associated with the AA-LTs signalling pathway.¹⁴¹ The presence of inflammation is usually associated with the transcriptional activation of NF- κ B. To this end, Horrillo et al examined the effect of LTB4 on NF- κ B activity,

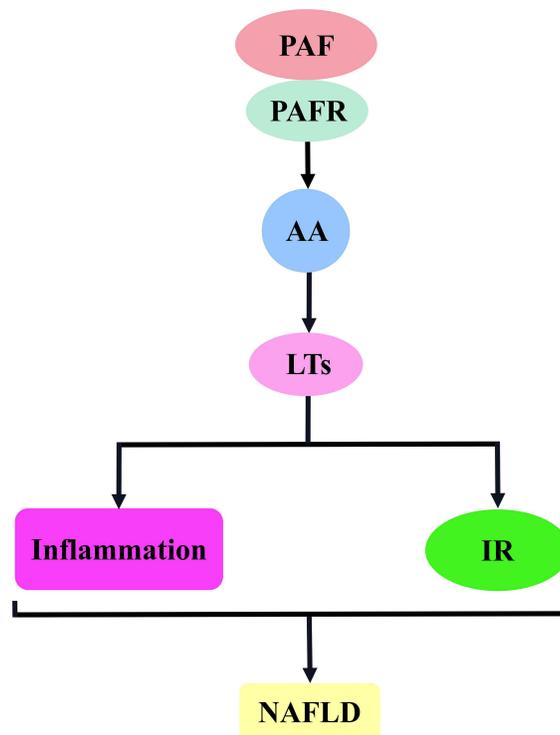


Figure 6 PAF activates the AA–LTs signalling pathway to promote the mechanism of NAFLD development. The binding of PAF to PAF receptor stimulates the release of AA, which induces LTs release and is involved in NAFLD development mainly by inducing inflammatory responses and insulin resistance.

Abbreviations: PAF, platelet-activating factor; PAFR, platelet-activating factor receptor; AA, arachidonic acid; LTs, leukotrienes; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease.

and their results showed that LTB₄ can induce NF- κ B activity in adipose tissue in a concentration-dependent manner. Given that NF- κ B activation stimulates the production of pro-inflammatory cytokines, further studies found that LTB₄ stimulates adipose tissue to produce the pro-inflammatory cytokines MCP-1, IL-6 and TNF- α , which would exacerbate adipose inflammatory damage.¹³⁸ Furthermore, IR may be another mechanism of LTs' involvement in NAFLD. This idea is supported by Spite et al who knocked out *BLT1* in mice on a HFD, resulting in significant improvements in glucose homeostasis and IR.¹⁴² Moreover, Abdallah et al used a cysteinyl-LT₁ receptor antagonist (montelukast), which significantly improved fasting blood glucose, fasting insulin level and IR in patients with NASH by blocking the pro-inflammatory effects of LTD₄.¹⁴³

PAF–AA–PG–TXA₂ Signalling Pathway and NAFLD

The PAF–AA–PG–TXA₂ signalling pathway is dependent on the activation of the PAF–AA signalling pathway; catalysed by COX, AA is converted to the unstable prostaglandin H₂ (PGH₂). After being induced by various synthases, PG and TXA₂ were formed. PG mainly includes PGI₂, PGD₂, PGE₂ and PGF₂ α .¹⁴⁴ This effect is present in platelets and various cells. For example, Son et al induced the production of PGD₂ and TXA₂ using AA-treated rabbit platelets.¹⁴⁵ Similarly, in Srivastava's experiments, human platelets showed significantly higher levels of PGF₂ α , PGE₂ and TXB₂ after AA treatment.¹⁴⁶ This effect is also present in hepatocytes. Levine used PAF to treat hepatocytes, which promoted the production of PGI₂, PGE₂ and PGF₂ α by inducing AA metabolism.¹⁴⁷ Similarly, Levine's incubation of rat hepatocytes using PAF or AA promoted the production of PGI₂.¹⁴⁸ The synthesis of PG and TXA₂ requires the catalysis of COX enzymes. This was confirmed in the experiments by López-Parra et al Significant expression of COX-1 and COX-2 in rat mesangial cells at rest induced the production of AA metabolites such as PGE₂, TXB₂ and 8-epi-PGF₂ α . The use of COX-2 inhibitor celecoxib down-regulated 8-epi-PGF₂ α and PGE₂ levels.¹⁴⁹ Similarly, Becker et al used PAF to stimulate neonatal rat cardiomyocytes to see if it could induce AA release. Their results showed a concentration-dependent release of PGI₂ and TXA₂. Interestingly, this effect was attenuated by the COX inhibitor aspirin

(acetylsalicylic acid) and the PAF antagonist Web2086.¹⁵⁰ Moreover, Lo et al used AA to induce platelet aggregation to investigate the mechanism of platelet aggregation inhibition by 2-ethoxy-5-methoxy-2-(5-methylthienyl) chalcone (EMMTC). Their results suggested that AA triggers platelet aggregation by inducing TXB₂ activation; however, EMMTC or indomethacin (COX inhibitors) abolished this process.¹⁵¹ Accordingly, PAF activates the AA metabolic pathway to produce various products, such as PG and TXA₂, and the PAF-AA-PG/TXA₂ signalling pathway is formed. PG and TXA₂ are both AA metabolites, and these derivatives have important regulatory roles in inflammatory responses, glucose metabolism and lipid metabolism, which are associated with the development and progression of many diseases, such as diabetes, hypertension and obesity.^{152–154}

Both PG and TXA₂ are downstream derivatives of AA, and the catalytic enzymes that induce AA conversion are mainly COX-1 and COX-2.^{144,155} In the animal model of NAFLD, in addition to increased AA levels, the expressions of COX-1 and COX-2 were significantly up-regulated.^{34,156} This suggests that PG and TXA₂ may have been involved in NAFLD development. Aspirin (acetylsalicylic acid) was originally used as an anti-inflammatory drug. It is also an irreversible inhibitor of the COX enzyme that produces PG and thromboxane precursors.¹⁵⁷ Interestingly, in recent years, the clinical use of aspirin has been effective in alleviating NAFLD tissue damage and reducing NAFLD prevalence and the risk of liver fibrosis.^{30–33} Moreover, in NAFLD animal models, aspirin application significantly improved hepatic steatosis and delayed NAFLD development.^{34–37} Aspirin is suggested to alleviate NAFLD, probably by inhibiting PG and TXA₂ syntheses. In summary, the induction of AA expression by PAF may have promoted the production of PG and TXA₂, which are further involved in NAFLD development.

Both PG and TXA₂ play an important role in the development of NAFLD. Of these, PG is primarily involved in the progression of NAFLD by regulating oxidative stress, affecting immune cells and interfering with glucose metabolism, whereas TXA₂ impairs insulin sensitivity, triggers inflammatory responses and aggravates hepatocyte damage (Figure 7).^{153,154,158}

AA, catalysed by COX enzymes, produces large amounts of PG, which drives NAFLD progression by acting on hepatocytes. This view is supported by Pérez et al, who used PGE₂, PGD₂ and PGF₂α to act directly on unstimulated primary rat hepatocytes. As a result, ApoB and lipid secretion are significantly reduced, resulting in decreased triacylglycerol (TAG) and cholesterol transport to the circulation. This may be a cause of steatosis.¹⁵⁹ Similarly, Henkel et al used PGE₂ to treat obese rat hepatocytes. Their results showed that PGE₂ decreased the expression of adipose triglyceride lipase, mitochondrial β-oxidation regulator carnitin-palmitoyltransferase 1 (*CPT-1*), apolipoprotein B (*ApoB*) and microsomal transfer protein, via promoted hepatocyte fat accumulation by inhibiting hepatic lipolysis, β-oxidation and very-low-density lipoprotein (VLDL) synthesis.¹⁶⁰ Consistent with cellular experiments, Nassir et al confirmed the relevance of PG to NAFLD in animal experiments, and they established a cluster of differentiation 36 (*Cd36*)-deficient mice to explore the role of the *Cd36* gene in hepatic VLDL secretion. Their results showed that *Cd36* gene deletion aggravated steatosis by increasing the expressions of PGE₂, PGD₂ and PGF₂α in the liver, impairing the secretions of triglyceride and ApoB in the liver.¹⁶¹ Moreover, clinical data confirm the correlation between PG and NAFLD. Henkel et al compared the expression of PGE₂ synthase (COX-2 and microsomal prostaglandin E synthase 1 [mPGES-1]) in liver samples from patients with NASH and healthy controls. Their results showed that COX-2 and mPGES-1 in the NASH group were much higher than those in the control group; moreover, they were significantly correlated with NASH activity score (NAS).¹⁶² Similarly, Loomba et al compared plasma eicosanoids in the NAFL group, NASH group and healthy group quantitatively, and their results showed that PGD₂ product levels were up-regulated in the NASH group, significantly correlated with NAFLD development and could even be used as a discriminatory marker between NAFL and NASH.¹⁶³

PG can be involved in NAFLD development in some ways, such as the inflammatory response. This was demonstrated in animal tests by Kus et al who used a HFD to induce an NAFLD mouse model. To explore the inflammatory response of liver sinusoidal endothelial cells (LSECs) in the early and late stages of NAFLD. Their results suggest that AA metabolism induces the activations of PGD₂ and PGI₂ via COX-1/2 early in NAFLD, further enhancing the anti-inflammatory response. In the late stage of NAFLD, AA metabolism produces pro-inflammatory factors through COX-2, including PGE₂ and PGF₂α, thus triggering inflammatory response signals. An imbalance in the expression of anti-inflammatory and pro-inflammatory factors will contribute to NAFLD progression.¹⁶⁴ In response, Kumei et al knocked

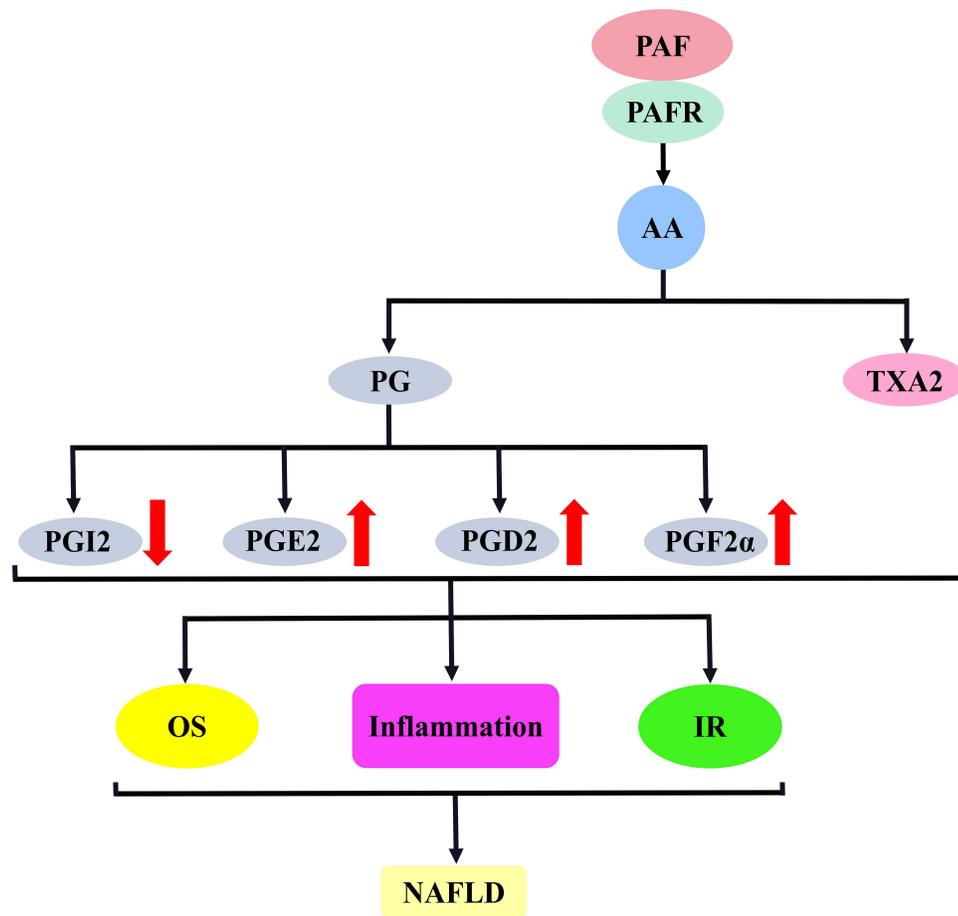


Figure 7 PAF activates the AA–PG/TXA2 signalling pathway to promote the mechanism of NAFLD development. The binding of PAF to PAFR stimulates AA release, which induces the synthesis of PG and TXA2; PG mainly included PGI2, PGE2, PGD2 and PGF2 α . These substances are involved in NAFLD development mainly through the induction of oxidative stress, inflammatory response and insulin resistance.

Abbreviations: PAF, platelet-activating factor; PAFR, platelet-activating factor receptor; PG, prostaglandins; TXA2, thromboxane A2; PGI2, prostaglandin I2; PGE2, prostaglandin E2; PGD2, prostaglandin D2; PGF2 α , prostaglandin F2 α ; OS, oxidative stress; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease.

out the mouse *Ip* gene, which promoted the expression of inflammatory factors (*Tnf- α* and *Mcp-1*) by blocking anti-inflammatory PGI2 signalling, leading to hepatocyte injury and driving NASH development. The use of IP-specific agonists (belaprost sodium) enhances PGI2 signalling and has good anti-NAFLD activity.¹⁶⁵ Furthermore, Chung et al used a HFD to feed rats, and their results showed that the up-regulation of COX-2 activity promoted inflammatory response-dependent NF- κ B and downstream inflammatory factor (*MCP-1* and *TNF- α*) expression through the induction of pro-inflammatory factor PGE2 expression, and these were significantly reversed by green tea extract by decreasing PGE2 expression.¹⁶⁶

Oxidative stress may be another mechanism by which PG is involved in NAFLD development. Chung et al used a HFD to feed rats, and their results showed that a HFD increased PGE2 expression through the up-regulation of COX-2 activity and elevated the MDA level, which represents the level of oxidative stress, suggesting that PGE2-mediated oxidative stress is involved in NAFLD development.¹⁶⁶ Interestingly, Kumei et al knocked out the *Ip* gene in mice and promoted the production of lipid peroxidation products (4-hydroxynonenal and thiobarbituric acid reactive substances) by blocking PGI2 signalling and activating oxidative stress, which contributed to NAFLD development. Therefore, the levels of PGI2 and PGE2 expressions may determine the level of oxidative stress in the liver.¹⁶⁵

PG may also mediate IR and be involved in NAFLD progression. Clinical data show that PG or PG metabolites are increased in patients with diabetes, including PGE2, PGI2 in islet or blood, 15-keto-dihydro-PGF2 α and 8-iso-PGF2 α .^{167–171} IR is closely related to diabetes development. PG may be also involved in IR development. In this regard,

Francés et al used a HFD to feed mice, resulting in significant hepatic COX-2 expression, which promoted PGE2 production and led to hepatic IR.¹⁷² Furthermore, Hsieh et al significantly down-regulated PGE2 metabolites and improved insulin sensitivity in rats on a HFD using COX-2 inhibitors.¹⁷³ Similarly, Henkel et al suggested that PGE2, as a COX product, may contribute to IR development. To this end, they incubated rat hepatocytes with PGE2 and insulin to investigate the role of PGE2 in hepatic IR. As a result, glycogen synthesis and AKT phosphorylation were inhibited. To clarify the possible mechanism of this effect, they pre-treated HepG2 cells expressing the EP3 receptor with PGE2. Accordingly, PGE2 induces phosphorylation of the insulin receptor substrate serine, possibly through activation of EP3 receptor-dependent extracellular signal-regulated kinase 1/2. This will lead to the inhibition of AKT phosphorylation and glycogen synthesis, thus promoting IR in the liver.¹⁷⁴ After an in-depth study, Henkel et al speculated that PGE2 may affect hepatic IR by regulating the release of other signalling molecules from Kupffer cells. To explore this possibility, they established a NASH rat model and used PGE2 to treat the isolated Kupffer cells. Their results showed that PGE2 stimulated Kupffer cells to produce oncostatin M, which may lead to the phosphorylation of the signal transducer and activator of transcription 3, thereby inducing the activation of the suppressor of cytokine signal transduction 3. Subsequently, this signalling cascade attenuates AKT activation, leading to blocked glucokinase expression, which contributes to IR and NAFLD development.¹⁷⁵ Interestingly, circulating PGF2 α was also significantly elevated in patients with diabetes. To this end, Wang et al established a PGF2 α receptor (FP)-deficient mouse model to investigate whether and how PGF2 α regulates hepatic glucose metabolism. Their results indicated that FP receptor deletion in mice inhibits hepatic gluconeogenesis and improves insulin sensitivity and glucose homeostasis. PGF2 α is suggested to induce hepatic gluconeogenesis via FP receptors and disrupts glucose homeostasis, which contributes to NAFLD development.¹⁷⁶ Moreover, Sato et al significantly improved the serum glucose and insulin levels in obese Zucker rats using the prostacyclin analogue belaprost sodium, suggesting that PGI2 is beneficial in alleviating IR in NAFLD.¹⁷⁷

AA generates TXA2 catalysed by COX enzymes, which can drive NAFLD by inducing IR. This hypothesis was confirmed by the animal experiments by Wang et al, who used a HFD to induce NAFLD in a mouse model. As a result, AA-dependent COX-1/2 expression was up-regulated and induced TXA2 release by enhancing thromboxane A2 synthase and thromboxane A2 receptor activities, which affected insulin sensitivity and glucose tolerance. This contributes to NAFLD development. Therefore, the pharmacological inhibition of COX-1/2 and thromboxane A2 receptor expression has a good effect on alleviating NAFLD.³⁴ Moreover, TXA2 can modulate the inflammatory response and drive NAFLD development. To this end, Ryu et al used a HFD-containing persimmon leaf extract fed to rats. As a result, a HFD induced the release of TXA2 or TXB2 and promoted the expression of inflammation-related factors, including TNF- α , C-reactive protein and leptin. This triggers an inflammatory response that drives the onset and progression of NAFLD. Persimmon leaf down-regulation of TXA2 and TXB2 expressions reversed this result.¹⁷⁸ However, TXA2 causing steatosis is the most direct evidence of involvement in NAFLD. This idea was supported by Francque et al who used methionine–choline-deficient (MCD) diet-induced steatosis rat model to explore steatosis-associated factors. Their results showed that the expression concentration of hepatic thromboxane synthase was significantly increased, suggesting that the increased production of TXA2 may be an important causative factor for steatosis-related diseases.¹⁷⁹ The dynamic balance of PGI2/TXA2 in vivo can maintain the stability of vascular microcirculation. Increased TXA2 or decreased PGI2 induces coagulation and thrombosis through excessive platelet activation. This will further aggravate hepatic microcirculatory disorders, thus promoting hepatocyte degeneration necrosis and fibrosis, which contribute to NAFLD development.¹⁸⁰

PAF–DAG Signalling Pathway and NAFLD

The PAF–DAG signalling pathway is activated by PAF by driving phospholipase C activation to produce DAG from phosphatidylinositol catabolism. Therefore, in preclinical studies, PAF has a strong activating activity for DAG. For example, Murphy et al used PAF to activate platelets, which resulted in the rapid increase in DAG levels.¹⁸¹ Furthermore, Catalán et al evaluated the effect of PAF on phosphatidylinositol hydrolysis in rat brain slices, and their results showed that PAF pre-treatment activated phospholipid inositol hydrolysis and induced DAG release in a dose-dependent manner.¹⁸² Similarly, Kester et al investigated the effect of PAF treatment on phosphatidylinositol hydrolysis, using glomerular thylakoid cells, and their results showed that PAF stimulated the accumulation of DAG.¹⁸³ Previously, Uhing

et al reported similar results in an assay involving PAF stimulated DAG accumulation in murine peritoneal macrophages. PAF activity was higher and induced PLC activation triggering DAG release.¹⁸⁴ In hepatocytes, this effect is also present. This was confirmed in the experiments by Okayasu et al who incubated rat hepatocytes with PAF and showed a down-regulation of phosphatidylinositol content and a progressive increase in DAG content.⁶⁷ Similarly, Miguel et al showed a rapid and dose- and time-dependent increase in the mass of DAG when PAF was used to treat isolated rat hepatocyte nuclei. However, WEB2086 or PCA-4248 (PAF receptor antagonist) abolished DAG synthesis.¹⁸⁵ Accordingly, the PAF–DAG signalling pathway was formed. DAG is an intermediate product of fat metabolism, a second messenger. It can regulate glucose intake and utilisation, as well as the storage and movement of fat and other life activities.¹⁸⁶

DAG is a lipid that may be deposited in hepatocytes as a result of PAF-induced abnormal expression, which leads to hepatocyte dysfunction or even injury. When DAG accumulates in large amounts in the liver, it may induce lipotoxicity and affect the glucose metabolism. Moreover, in such cases, the liver, as an insulin-target organ, acts as an IR, which helps to promote NAFLD development.¹⁸⁷ In this regard, Preuss et al used liquid chromatography triple quadrupole mass spectrometry to analyse the DAG of the NAFLD mouse model, and their results showed significantly up-regulated type and concentration of DAG.¹⁸⁸ Similarly, Gorden et al used mass spectrometry liposomes to analyse DAG changes during NAFLD progression. Significant differences in DAG species were confirmed between the normal and diseased livers of humans and mice.¹⁸⁹ In addition, Sanyal et al analysed the changes in liver lipid components, such as DAG and cholesterol, over time in a diet-induced NAFLD model, using a lipidomic approach. Interestingly, the content of monounsaturated fatty acids containing DAG and cholesterol esters was significantly increased along with fatty liver, inflammation and swelling.¹⁹⁰ Earlier literature discussed the hypothesis that DAG mediates IR and induces type 2 diabetes (T2D) and NAFLD.¹⁹¹ The reason for this is an excessive accumulation of DAG in hepatocytes, which induces lipotoxicity and impairs hepatic insulin sensitivity.^{187,192} This was confirmed by Magkos et al who evaluated the effect of hepatic DAG content on insulin sensitivity in patients with obesity. They found that intrahepatic DAG content is positively correlated with steatosis and NAFLD activity scores, suggesting that DAG aggregation causes hepatic lipotoxicity, and that insulin's inhibitory effect on hepatic gluconeogenesis is negatively correlated with DAG, suggesting that lipotoxicity impairs insulin sensitivity.¹⁹³ In the liver, insulin plays a role in promoting glycogen synthesis—a process regulated by glycogen synthase—in addition to inhibiting glucose production.^{194,195} In this regard, Blackmore et al incubated hepatocytes using exogenous phospholipase C, which resulted in a time-dependent accumulation of DAG and inactivation of hepatic glycogen synthase, indicating that DAG may promote IR by inhibiting hepatic glycogen synthesis.¹⁹⁶ Under normal conditions, insulin binds to specific receptors on the surface of hepatocytes, and tyrosine kinases in the receptors are activated to induce trans-autophosphorylation of the tyrosine residues. This further confers catalytic activity to the insulin receptor; recruits to catalyse tyrosine phosphorylation of multiple proteins, including insulin receptor substrate (IRS); activates the downstream PI3K/AKT and other pathways; and ultimately regulates the hepatic glucose metabolism.^{197–199} To elucidate the specific molecular mechanisms of DAG-induced hepatic IR, Lyu et al used antisense oligonucleotides (ASO) to reduce the rat liver DGAT2 protein, which is an enzyme that catalyses the esterification of DAG for producing triglycerides, thereby leading to a significant increase in rat liver DAG content and reducing the phosphorylation level of insulin receptor kinase (IRK)-T1162; this is accompanied by impaired phosphorylation of the downstream insulin-stimulated AKT-S473, glycogen synthase kinase GSK3 β -S9 and glycoisomerisation inhibitor enzyme FOXO1-S256.²⁰⁰ In addition, Aroor et al fed a high-fat and high-sugar diet to mice who showed a significant increase in hepatic DAG content, resulting in impaired hepatic insulin sensitivity by reducing AKT phosphorylation, which reduced the effect of insulin inhibition on hepatic glucose output.²⁰¹ This finding suggests that the down-regulation of hepatic DAG content helps improve insulin sensitivity. This was confirmed by the experiments of Li et al who provided lipocalin treatment to mice on a HFD, which significantly reduced hepatic DAG levels, promoted insulin receptor tyrosine 1162 phosphorylation, increased hepatic IRS-2-associated PI3K kinase activity and AKT-serine phosphorylation levels, improved hepatic insulin sensitivity and increased the inhibitory effect of insulin on hepatic glucose production.²⁰² A hypothesis suggests that a link between hepatic DAG accumulation and IR may be attributed to PKC activation.²⁰³ In this regard, Gilijamse et al analysed liver biopsies from patients who were obese, and their results showed increased hepatic cytoplasmic DAG content and PKC ϵ translocation to the plasma membrane in patients with hepatic insulin resistance, suggesting the relevance of hepatic DAG-induced PKC ϵ activation in the pathogenesis of

NAFLD-associated hepatic IR.²⁰⁴ Similarly, Kumashiro et al used liver biopsy tissue from patients who were obese and without diabetes and correlated liver and plasma markers with steady-state model assessments of IR indices. Their results showed that the DAG content in hepatocyte plasma lipid droplets was the best predictor of IR; in addition, liver tissue DAG content was positively correlated with hepatocyte PKC ϵ activation, suggesting that DAG-mediated PKC ϵ is involved in hepatic IR.²⁰⁵ In summary, DAG may contribute to NAFLD development.

PAF–DAG–PKC- ϵ /PKC- δ Signalling Pathway and NAFLD

The PAF–DAG–PKC- ϵ /PKC- δ signalling pathway is dependent on the activation of the PAF–DAG signalling pathway, and DAG further induces the activation of downstream PKC and its congeners (PKC- ϵ , PKC- δ).^{206,207} Therefore, PAF had strong agonistic activity against PKC in preclinical studies. This was confirmed in the experiments by Pelech et al. They exposed rabbit platelets to PAF and showed that PAF-induced a significant increase in PKC kinase activity.²⁰⁸ Similarly, Gay et al exposed human neutrophils to PAF and showed a significant increase in PKC activity associated with the granule fraction compared with control cells.²⁰⁹ Furthermore, in the experiments by Hu et al, PAF released from leukocytes enhanced PKC activity, induced platelet activation and promoted platelet P-selectin expression. This effect is time-dependent. Interestingly, SR121566 and C7E3 (GPIIb/III α inhibitor) abolished PKC release, suggesting that platelet activation may be associated with glycoprotein IIb/III α (GPIIb/III α).²¹⁰ Similarly, Guo et al reported similar results in a study involving PAF-induced inflammatory damage of human bronchial smooth muscle. Elevated PAF activity triggered PKC protein expression and exacerbated bronchial smooth muscle inflammatory injury. However, hydroxysafflor yellow A abolished this effect, probably because of its PAFR antagonist.²¹¹ Notably, DAG is a key link in the activation of PKC- ϵ and PKC δ .²¹² This hypothesis has been confirmed in several experiments. For example, Sharma et al performed a trial involving the inflammatory response of adipocytes. The study showed that pre-treatment with the DAG analogue 1-Oleoyl-2-acetyl-sn-glycerol would induce PKC- δ activation in adipocytes and further stimulate downstream pro-inflammatory gene expression.²¹³ Moreover, similar results were reported by Balciunaite et al in a trial involving PDGF-activated PKC, and PDGF-pre-treated HepG2 cells with PKC- ϵ content were up-regulated over time after the addition of DAG.²¹⁴ Based on this, PAF induces PKC- ϵ and PKC- δ activation via DAG, and the PAF–DAG–PKC- ϵ /PKC- δ signalling pathway is formed. PKC is an important intracellular signal transduction molecule that regulates cell motility, adhesion, proliferation, differentiation and apoptosis, and PKC- ϵ and PKC- δ belong to new isoforms of PKC that regulate activities, such as glucose metabolism, inflammation and apoptosis, and are associated with disease progression.^{215–218}

PKC is a lipid-activated signalling molecule; and its subtypes, PKC- ϵ and PKC δ , demonstrate a high affinity for DAG.^{219,220} PKC- ϵ induces NAFLD by the mechanism of insulin receptor down-regulation and lipid accumulation,²²¹ and PKC δ is closely related to multiple signalling pathways, such as oxidative stress, inflammatory response, ERS and pro-apoptosis, in addition to impaired insulin sensitivity, which plays a key role (Figure 8).^{222–224}

In hepatocytes, DAG-induced activation of PKC- ϵ and PKC δ promoted NAFLD development, possibly through regulation of IR. This idea was confirmed by Kumashiro et al who used liver biopsy tissue from patients who were obese and without diabetes to analyse the association of liver and plasma markers with IR. Their results showed that DAG content in hepatocyte plasma lipid droplets was the best predictor of IR; in addition, liver tissue DAG content was positively correlated with hepatocyte PKC ϵ activation, suggesting that DAG-mediated PKC ϵ is involved in hepatic IR.²⁰⁵ Similarly, to examine the role of PKC δ in cellular IR, Greene et al isolated primary hepatocytes from HFD mice and stimulated them with insulin. Primary hepatocytes from PKC δ -deficient mice improved insulin-stimulated AKT compared with hepatocytes from wild-type mice.²²⁵ In line with this, DAG regulation of PKC- ϵ and PKC δ expression, induction of IR and involvement in NAFLD development were also confirmed in animal experiments. Jornayvaz et al assessed the role of DAG in causing liver IR using diacylglycerol acyltransferase 2 overexpressing mice. Their results showed that a significant increase in hepatic DAG down-regulated IRS-2 tyrosine phosphorylation levels through PKC ϵ activation, leading to IR.²²⁶ This is similar to the results of the experiment by Dallak who fed rats with acylated ghrelin, resulting in an up-regulation of hepatic DAG content, leading to PKC- ϵ and PKC- δ activation, promoting IRS (Ser307) phosphorylation and inducing hepatic IR.²²⁷ Conversely, the inhibition of PKC ϵ or PKC- δ signalling improves IR, which helps alleviate NAFLD. For instance, Frangioudakis et al evaluated the effect of *Pkc- δ* and *Pkc- ϵ* gene deletion on

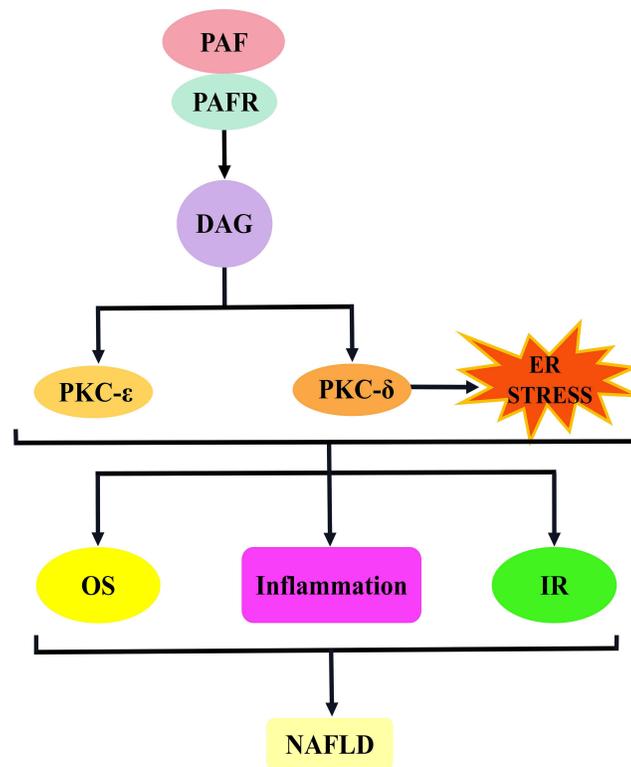


Figure 8 PAF activates the DAG–PKC ϵ /PKC δ signalling pathway to promote the mechanism of NAFLD development. The binding of PAF to PAFR induces DAG production. The activation of DAG induces PKC (PKC ϵ and PKC δ) activation, and PKC δ may also agonise endoplasmic reticulum stress. This pathway is involved in NAFLD development mainly through the induction of oxidative stress, inflammatory response and insulin resistance.

Abbreviations: PAF, platelet-activating factor; PAFR, platelet-activating factor receptor; DAG, diacylglycerol; PKC, protein kinase C; PKC ϵ , protein kinase C ϵ ; PKC δ , protein kinase C δ ; OS, oxidative stress; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease.

abnormal glucose tolerance due to a HFD in a controlled trial in wild-type mice. Their results showed improvements in glucose tolerance, insulin sensitivity and blood glucose levels.²²⁸ This result was supported by Zhang et al, who established *Pkc- δ* -silenced mice to analyse the changes in insulin signalling. By contrast, the phosphorylation levels of AKT and GSK3 β in the liver of *Pkc- δ* -silenced mice were increased, and the liver IR was relieved.²²⁹ Analogously, Samuel et al reported similar results in a trial to discern whether PKC ϵ was associated with hepatic IR or not, and they used antisense oligonucleotides targeting PKC ϵ to down-regulate *Pkc- ϵ* expression and reverse the defective hepatic insulin signalling.²³⁰

In addition to IR, PKC- δ can be involved in NAFLD development in other ways. Greene et al compared the indexes of oxidative stress, inflammation and apoptosis between *Pkc- δ* -deficient mice and wild-type mice induced by MCD diet to explore the ways of PKC- δ in promoting NASH development. As a result, the inflammation score and NADPH oxidase activity were significantly reduced in *Pkc- δ* -deficient mice, and the expressions of apoptotic genes *caspase-3* and *caspase-9* were inhibited, suggesting that the abnormal expression of PKC- δ is involved in the regulation of inflammation, oxidative stress and apoptosis in various aspects, driving NAFLD development.²³¹ Similar to some of the observations, Klymenko et al evaluated the effect of *PKC- δ* gene silencing using siRNA on endothelial function in diabetic vascular smooth muscle cells using a streptozotocin-induced diabetic rat model. Their results suggest that *PKC- δ* silencing inhibits oxidative stress-dependent ROS production.²³² Interestingly, Pereira et al further investigated that PKC- δ induced IR may be causally related to oxidative stress. They used heparin infusion to up-regulate FFAs and established IR rats, resulting in elevated hepatic PKC- δ levels, along with the detection of a large number of NADPH oxidase and oxidative stress markers and increased c-JNK expression. Interestingly, antioxidants and NADPH oxidase inhibit JNK expression, thereby attenuating IR. PKC- δ is suggested to induce JNK activation through oxidative stress, which contributes to IR development.²³³ This view is supported by the clinical study of De et al, who determined insulin

sensitivity before and after intravenous administration of glutathione with antioxidant effects in 10 patients with non-insulin-dependent diabetes mellitus and 10 healthy individuals. Their results showed a significant improvement in insulin-mediated systemic glucose uptake.²³⁴

Evidence shows that ERS plays an important role in the pathological changes of NAFLD.²³⁵ For this reason, Lai et al used PA to induce the L02 cell steatosis model to explore whether ERS is another mechanism of PKC- δ promoting NAFLD development. They found that the silent *PKC- δ* gene can alleviate PA-induced ERS. In addition, siRNA can inhibit the activity of the *PKC- δ* gene, reduce intracellular calcium overload and restore the function of SERCA, a calcium balance regulator.⁸⁵ Similarly, Yang et al established a liver ERS model in L02 cells induced by FFA. Similar conclusions were obtained by studying the effect of PKC- δ silencing on ERS: PKC- δ down-regulated SERCA activity, destroyed calcium homeostasis and induced ERS, which was helpful to NAFLD development.²³⁶ This conclusion was echoed by Greene et al, who used MCD diet to establish a mouse model of NASH to investigate the causal relationship between PKC- δ and ERS. Their results showed that the PKC- δ protein content was consistent with ERS expression parameters. In vitro induction experiments with the help of MCA cells confirmed that *PKC- δ* gene silencing down-regulated the expression of ERS markers CHOP and protein kinase-like ER-resident kinase.²³⁷

PAF–DAG–p38MAPK Signalling Pathway and NAFLD

The PAF–DAG–p38MAPK signalling pathway depends on the activation of the PAF–DAG signalling pathway, and DAG further stimulates p38MAPK activation. Therefore, PAF demonstrated a strong agonistic activity on p38MAPK in preclinical studies. For example, Wang et al conducted an experiment involving PAF regulating the proliferation and differentiation of colon cancer cells, and their results showed that PAF preconditioning triggered the activation of p38MAPK and induced the growth inhibition and differentiation of colon cancer cells.²³⁸ In addition, Yu et al confirmed that this effect also exists in microvessels. They treated mesenteric adipose tissue of mice with PAF and measured microvasculature transport using a computer program. Their results showed that PAF up-regulated microvascular permeability. Interestingly, SB203580 (p38MAPK inhibitor) reversed this effect. This suggests that p38MAPK is a regulatory element in the signalling cascade of PAF-induced microvascular permeability.²³⁹ In fact, the mechanism of PAF activating p38MAPK through DAG is unknown. Evidence shows that oxidative stress is an important factor of p38MAPK activation.²⁴⁰ More importantly, DAG-induced PKC expression facilitates ROS production.^{241,242} Thus, DAG may activate p38MAPK through ROS. Based on the above, the PAF–DAG–p38MAPK signalling pathway is formed. p38MAPK is a member of the mitogen-activated protein kinase (MAPK) family. As a signal transduction molecule, p38MAPK plays a key role in regulating insulin signalling, sugar transport, inflammation and apoptosis.^{243–245}

p38MAPK is an important inflammatory transcription factor that induces the release of inflammatory factors, initiates cellular damage mechanisms and plays a key role in the development of NAFLD. In addition, the activation of p38MAPK can affect insulin signalling and further aggravate NAFLD injury (Figure 9).^{246,247}

DAG facilitates ROS generation, and ROS may induce hepatic IR via p38MAPK, which contributes to NAFLD development. This hypothesis was confirmed by Gao et al. They used dairy cow hepatocytes and HepG2 cells with non-esterified fatty acids (NEFA) to examine the relationship between mitochondrial dysfunction and IR. Their results showed that NEFA activated p38MAPK by up-regulating ROS content and reduced the phosphorylation levels of IRS-2 and AKT, thus inducing IR. Interestingly, antioxidants lipoic acid and SB203580 (p38MAPK inhibitors) reversed this effect.²⁴⁸ These results were supported by Liu et al. They fed rats with a HFD to explore the mechanism of JLD improving insulin sensitivity in IR rats. Studies have shown that a HFD can increase IRS-1 serine phosphorylation, decrease AKT phosphorylation and impair insulin signal transduction by activating p38MAPK. However, JLD reversed IR by down-regulating the expression of ROS, reducing p38MAPK-dependent oxidative stress.²⁴⁹ Zhu et al reported similar results. They adopted HepG2 cells with insulin and established an IR model to study the regulatory effects of pea-derived peptides on IR. Their results showed that pea-derived peptides probably blocked p38MAPK phosphorylation by inhibiting ROS, further reduced IRS-1 Ser307 phosphorylation, promoted Ser473 phosphorylation of AKT and improved IR.²⁵⁰

p38MAPK can regulate inflammatory response and promote NAFLD development. Therefore, Gong et al induced the NASH rat model with a HFD to explore the role of inflammation-dependent toll-like receptor 4 (TLR4)–p38MAPK

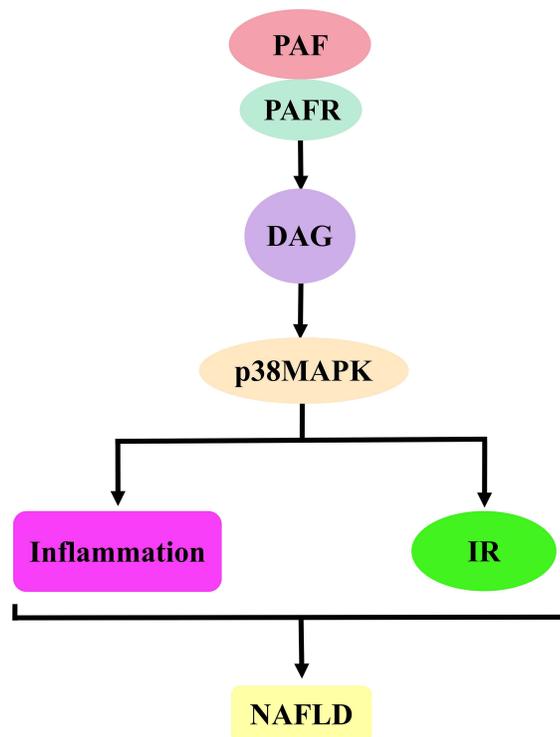


Figure 9 PAF activates DAG–p38MAPK signalling pathway to promote the mechanism of NAFLD development. The binding of PAF to PAFR induces DAG production. The activation of DAG induces p38MAPK activation, which is involved in NAFLD development by inducing inflammatory responses and IR.

Abbreviations: PAF, platelet-activating factor; PAFR, platelet-activating factor receptor; p38MAPK, p38-mitogen-activated protein kinases; OS, oxidative stress; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease.

signalling pathway in Kupffer cells and the intervention effect of the Shugan Jianpi recipe. Their results showed that a HFD induced *p38MAPK* activation through *TLR4* and promoted the release of inflammatory cytokines, including $TNF-\alpha$, *IL-1* and *IL-6*, which could trigger an inflammatory response and promote NAFLD development. The Shugan Jianpi recipe may reverse this effect by inhibiting the *TLR4*–*p38MAPK* signalling pathway.²⁵¹ These results were supported by Zhang et al They used FFA to induce human hepatocyte line L02 to establish a steatosis model and investigate the role of AMP-activated protein kinase $\alpha 1$ (*AMPK $\alpha 1$*) overexpression. Their results have shown that *p38MAPK* can enhance the inflammatory response by increasing pro-inflammatory cytokines (*IL-6*, *IL-12* and *TNF- α*) and decreasing anti-inflammatory cytokines (*IL-4*, *IL-10* and *IL-13*), which is helpful to NAFLD development. However, *AMPK $\alpha 1$* overexpression may inactivate *p38MAPK* and reduce the inflammatory response.²⁵² Notably, *p38MAPK* expression leads to the activation of many downstream inflammatory regulatory factors, including *COX-2*^{253,254} and *NF- κ B*,^{255,256} which can trigger and maintain the pro-inflammatory response and promote NAFLD development.

Summary and Prospect

NAFLD is a clinicopathological syndrome mediated by multiple factors, which has been attracting increasing attention because of its increasing prevalence and many hazards. The pathogenesis of NAFLD is extremely complex, and the widely recognised “two-hit” theory can only reflect the tip of the iceberg of the disease. Therefore, we clarified the mechanism of the relationship between the signalling pathway of platelet activation induced by PAF and NAFLD (Figure 2) through literature sorting, further enriching the pathogenesis of NAFLD and providing a theoretical basis for the clinical prevention and treatment of NAFLD. Although there is no specific drug for the treatment of NAFLD at present, many studies have shown that drugs that resist platelet activation, such as aspirin, ticlopidine and cilostazol, have a certain effect in delaying NAFLD progression, which also suggests that PAF plays a certain role in NAFLD development. The deficiency of this paper lies in that no literature related to the direct inhibition of PAF by antiplatelet

drugs to alleviate NAFLD was found. It only provides a new idea to improve the pathogenesis of NAFLD and find effective intervention measures.

Funding

This investigation was supported by a grant from Yunnan Province Applied Basic Research Project (2019FF002 (-055)).

Disclosure

The authors report no potential conflicts of interest related to this article.

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