Production of extracellular lysophosphatidic acid in the regulation of adipocyte functions and liver fibrosis

Fang Yang, Guo-Xun Chen

Fang Yang, School of Laboratory Medicine, Hubei University of Chinese Medicine, Wuhan 430065, Hubei Province, China

Guo-Xun Chen, Department of Nutrition, University of Tennessee at Knoxville, Knoxville, TN 37996, United States

ORCID number: Fang Yang (0000-0002-7092-2532); Guo-Xun Chen (0000-0001-6226-4050).

Author contributions: Yang F searched the publications, summarized data in tables, drew figures, discussed the literature and wrote the review; Chen GX designed the structure of the review and modified the review; all authors approved the final version of this review.

Supported by the National Natural Science Foundation of China, No. 31401510.

Conflict-of-interest statement: We declare that we have no conflicts of interest.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Manuscript source: Invited manuscript

Accepted: May 6, 2018
Article in press: May 6, 2018
Published online: September 28, 2018

Abstract

Lysophosphatidic acid (LPA), a glycerophospholipid, consists of a glycerol backbone connected to a phosphate head group and an acyl chain linked to sn-1 or sn-2 position. In the circulation, LPA is in submillimolar range and mainly derived from hydrolysis of lysophosphatidylcholine, a process mediated by lysosphospholipase D activity in proteins such as autotaxin (ATX). Intracellular and extracellular LPAs act as bioactive lipid mediators with diverse functions in almost every mammalian cell type. The binding of LPA to its receptors LPA1-6 activates multiple cellular processes such as migration, proliferation and survival. The production of LPA and activation of LPA receptor signaling pathways in the events of physiology and pathophysiology have attracted the interest of researchers. Results from studies using transgenic and gene knockout animals with alterations of ATX and LPA receptors genes, have revealed the roles of LPA signaling pathways in metabolic active tissues and organs. The present review was aimed to summarize recent progresses in the studies of extracellular and intracellular LPA production pathways. This includes the functional, structural and biochemical properties of ATX and LPA receptors. The potential roles of LPA production and LPA receptor signaling pathways in obesity, insulin resistance and liver fibrosis are also discussed.

Key words: Autotaxin; Lysophosphatidic acid receptors; Obesity; Lysophosphatidic acid; Insulin resistance; Liver fibrosis

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.
Lysophosphatidic acid (LPA) is mainly derived from hydrolysis of lysophosphatidylcholine, a process mediated by lysophospholipase D activity in proteins such as autotaxin (ATX). The binding of LPA to its receptors LPA1-6 activates multiple cellular signaling pathways and leads to changes. Studies using genetically modified animals have begun to reveal the roles of LPA pathways in metabolic active tissues and organs. The present review summarized recent progresses in the studies of extracellular and intracellular LPA production pathways; the functions, structural and biochemical properties of ATX and LPA receptors. Furthermore, the potential roles of LPA production and LPA receptor signaling pathways in obesity, insulin resistance and liver fibrosis are discussed.

Yang F, Chen GX. Production of extracellular lysophosphatidic acid in the regulation of adipocyte functions and liver fibrosis. World J Gastroenterol 2018; 24(36): 4132-4151 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i36/4132.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i36.4132

INTRODUCTION

Lysophosphatidic acid (1- or 2-acyl-sn-glycerol 3-phosphate/acyl-glycerol-phosphate, LPA) is one type of water-soluble glycerophospholipid with molecular mass about 430-480 Da. All LPA molecules contain a glycerol backbone linked to a phosphate head group at sn-3 position and an acyl chain esterified to sn-1 or sn-2 position. Due to variation of the fatty acyl chain, LPA molecules are in different forms and derived from multiple sources, such as membrane lipids as signaling mediators and membrane components, biological effects of LPA have drawn attention of biomedical scientists.

Subsequently, LPA in incubated serum at 36°C for 18-24 h was shown to cause aggregation of feline and human platelets. Whether LPA acts through its detergent-like physical property or its interaction with a specific receptor remained a critical question. Later, LPA was shown to stimulate cell proliferation in a pertussis toxin-sensitive manner. This finding suggested that LPA acts through G protein-coupled receptor (GPCR). This information led to the cloning and identification of a GPCR, which is now known as LPA receptor 1 (LPA1). It is known now that as a bioactive lipid mediator, LPA activates at least 6 specific GPCRs, named as LPA1-6. These GPCRs are coupled with several Gα proteins such as Gα12/13, Gαq/11, Gα16/19, and Gαi. The binding of LPA to these receptors stimulates the activations of small GTPases, Ras, Rho, and Rac, and induces downstream actions.

The existence of extracellular LPA indicates its production outside a cell. Since autotaxin (ATX) was first identified from human plasma and found to be a lysophosphatic acid-producing enzyme in 2002, the ATX-LPA receptor signaling pathway has been implicated in a variety of disease processes including the vascular and neural development, hair follicle development, tumor progression, lymphocyte trafficking, bone development, pulmonary fibrosis, fat mass regulation, cholestatic pruritus, neuropathic pain, embryo implantation, obesity and glucose homeostasis, spermatogenesis, fetal hydrocephalus, chronic inflammation, cellular proliferation, and smooth muscle contraction during development. Both ATX and LPA have attracted the interest of researchers in an effort to understand their roles in pathophysiology and to develop new agents to treat above-mentioned pathological conditions.

EXTRACELLULAR AND INTRACELLULAR PRODUCTION AND DEGRADATION OF LPA

LPA and its common precursor lysophosphatidylcholine (LPC) can be found both extracellular and intracellular as signaling mediators and membrane components, respectively. Structurally, LPA is an acyl group esterified to the sn-1 or sn-2 position of the glycerol backbone. Due to the differences of acyl chain length, saturation and backbone position, various LPA chemical forms can be found in tissues and cells. Extracellular LPA is thought to mediate bioactive effects through LPA receptors. Intracellular LPA is an important intermediate for the de novo biosynthesis of complex glycerolipids, including monos-, di-, and triglycerides, as well as PLs. In addition, it has been thought that LPA can function as a ligand for transcription factor peroxisome proliferator-activated receptor γ (PPARγ)21. This indicates that LPA may play important roles in the regulation of gene expression.
Pathways for LPA production

As shown in Figure 2, there are five major pathways for LPA production, (1) the lysophospholipids-ATX (LPLs-ATX) pathway, (2) the phosphatidic acid - phospholipase A₁ or A₂ (PA-PLA₁/PLA₂) pathway, (3) the de novo glycerophosphate acyltransferase (GPAT) synthesis pathway, (4) the monoacylglycerol kinase (MAGK) pathway, and (5) the oxidative modification of low-density lipoprotein (LDL) pathway. Despite recent advances in the identification of the enzymes responsible for LPA production, the regulation of these enzymes still remains obscure.

LPLs-ATX pathway

In the first pathway, LPLs generated from PLs by PLA₁ or PLA₂ are converted to LPA by a plasma enzyme ATX[22,13], which we will describe in later part of this article. A major source of extracellular LPA is LPC, other LPLs such as lysophosphatidylserine and lysophosphatidylethanolamine can also be enzymatically processed to produce LPA. This pathway accounts for the majority of circulating LPA.

PA-PLA₁/PLA₂ pathway

LPA is also produced intracellularly as an intermediate for the synthesis of other glycerolipids[20]. LPA can be produced enzymatically from intracellular organelles such as mitochondria and endoplasmic reticulum. Phosphatidic acid (PA) is first generated from PLs or diacylglycerol by phospholipase D enzymes (PLD₁ and PLD₂) and diacylglycerol kinase (DGK) activities, respectively. Then, one acyl group is removed from the sn-1 position by PLA₁ or at the sn-2 position by PLA₂ enzymes to generate LPA. This pathway may be more important in specific tissues with expression of DGK such as the brain and skin[23].

De novo GPAT synthesis pathway

GPATs catalyze the first step in glycerolipid synthesis, i.e., the conversion of glycerol-3-phosphate (G3P) to LPA by the transfer of fatty acids from acyl-CoA. Since GPAT exhibits the lowest specific activity of enzymes in the de novo triacylglycerol (TAG) and PLs synthesis pathways, it has been considered to be the rate limiting enzyme for them[24]. Many studies have been published on the regulation of TAG synthesis and its relevance to obesity and insulin resistance. GPAT activity in mitochondria was shown to be regulated by fatty acid-binding protein (FABP)[25,26]. It has been shown that mitochondrial GPAT activity was inhibited by LPA. FABP reversed the inhibition of LPA through the binding and extracting LPA from the mitochondrial outer membrane. The extracted LPA was converted to PA by microsomes, where acylglycerophosphate acyltransferases (AGPATs) are located[25,26]. These results suggested that FABP regulated the de novo synthesis of PA through the stimulation of mitochondrial GPAT and transport of LPA from mitochondria to microsomes.

MAGK pathway

Lipid phosphate phosphatases (LPPs) are also involved in the LPA turnover: LPPs can be found extracellularly...
or intracellularly in endoplasmic reticulum or Golgi, where they dephosphorylate LPA, which leads to the formation of monoacylglycerol (MAG)[27]. MAG may then be phosphorylated by MAGK and thus participate in another round of LPA signaling[20]. Thus, the production of LPA is regulated by the availability of precursors as well as the expression of catalytic enzymes.

**Oxidative modification of LDL**

LPA was found as an active molecule on oxidized and modified LDL, in where it may contribute to platelet activation, endothelial cell stress-fiber and gap formation[28,29]. LPAs on these lipoproteins activate platelets through G-protein coupled LPA receptors and a Rho/Rho kinase signaling pathway, which leads to platelet shape change and subsequent aggregation. The biologically active LPA-like products generated by this non-enzymatic oxidation co-migrated with an authentic LPA standard in thin layer chromatography. LPA was found to be accumulated in atherosclerotic plaques, which might act to activate platelets. The level of LPA is very high in the human carotid atherosclerotic lesion, suggesting the roles in thrombogenesis and rupture[28,29].

An alternative explanation to the generation of LPA from oxidized LDL is that ATX might be activated. The acyl/alkyl composition, the precursor of LPA and the mechanism responsible for LPA generation in the oxidized LDL remain to be addressed in the future.

**Figure 2 Biochemical pathways of lysophosphatidic acid synthesis and degradation.** LPA can be produced extracellularly and intracellularly as signaling mediators and membrane components, respectively. There are five major pathways for LPA production, (1) the lysophospholipids-ATX (LPLs-ATX) pathway, (2) the phosphatic acid - phospholipase A1 or A2 (PA-PLA1/PLA2) pathway, (3) the de novo glycerophosphate acyltransferase (GPAT) synthesis pathway, (4) the monoacylglycerol kinase (MAGK) pathway, and (5) the oxidative modification of low-density lipoprotein (LDL) pathway. In the upper right corner of the figure, there are catalytically active isoforms (ATXα, ATXβ, ATXγ, ATXd and ATXe), which are expressed in different tissues. PLs: Phospholipids; PLA1/PLA2: Phospholipase A1/2; LPLs: Lysophospholipids; ATX: Autotaxin; ATXα-δ: Protein structure scheme of the domains of ATX; LPA: Lysophosphatidic acid; DAG: Diacylglycerol; DGK: Diacylglycerol kinase; PLD: Phospholipase D; PA: Phosphatidic acid; AGPAT: Acylglycerophosphate acyltransferase; MAG: Monoacylglycerol; MAGK: Monoacylglycerol kinase; LPP: Lipid phosphate phosphatase; G3P: Glycerol-3-phosphate; lysoPL: Lysophospholipase; GPAT: Glycerophosphate acyltransferase; LDL: Low-density lipoprotein; i: Intramembrane domain; SMB: N-terminal somatomedin B-like domains; L1: L1 linker region; PDF: Phosphodiesterase domain; L2: L2 linker region; NUC: C-terminal nuclease-like domain; LPAα: Lysophosphatidic acid receptor 1-4.
**Pathways for the degradation of LPA**

There are three major pathways that degrade LPA as shown in Figure 2. The first is the removal of phosphate to form MAG by LPPs. LPA has a half-life of 3 min when it is added to cells expressing LPP. Four isoforms of LPP have been cloned and characterized in mammals, LPP1/PAP-2α, LPP1/PAP-2γ, LPP2/PAP-2β, and LPP3/PAP-2β. The second LPA degradation pathway involves the action of AGPAT enzymes, which are known as lysophosphatidic acid acyltransferases. These microsomal enzymes catalyze the transfer of an acyl group from acyl-CoA to LPA to form PA. Proteins with AGPAT activity include a family of transmembrane enzymes, and membrane-associated proteins involved in membrane fission such as endophilin and C-terminal-binding protein/brefeldin A-ADP ribosylated substrate. The third pathway for LPA degradation involves the hydrolysis of the acyl group from the G3P head group by the action of lysophospholipases. The majority of characterized lysophospholipases act on LPC.

**ECTO-NUCLEOTIDE PYROPHOSPHATASE/PHOSPHODIESTERASES**

The ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP) family contains seven members with structurally similar catalytic domains that hydrolyze phosphodiester bonds in various substrates, including nucleoside triphosphates, LPLs, and choline phosphate esters. ATX, or ENPP2, is the best-characterized member of ENPP family. ENPPs are defined by their ability to hydrolyze phosphodiester bonds of various nucleotides in vitro. ATX/ENPP2 was originally identified as a tumor cell-motility-stimulating factor from the conditional medium of A2058 human melanoma cells. Since the addition of pertussis toxin reduced cellular motility, ATX’s effects were thought to involve Gαi/o-mediated signaling. ATX can be secreted as a 100 kDa glycoprotein. It is produced by multiple tissues including adipose tissue and brain. It is believed that the circulating ATX/ENPP2 is degraded by the liver. Extracellular LPA was found to be present in sub-micromolar ranges. The responsible enzyme was identified to be ATX. ATX-mediated autocrine signaling induces cell motility through LPA production and Gαi/o-mediated LPA receptor signaling.

From both a structural and evolutionary point of view, ENPP family members have been categorized into two subgroups, ENPP1-3 and ENPP4-7. ENPP1-3 all have two N-terminal somatomedin B-like (SMB) domains, a central phosphodiesterase (PDE) domain and a C-terminal nuclease (NUC)-like domain as shown in Figure 2. ENPP4-7 only have similarity in the PDE domain. The crystal structures of mouse and rat ATX show loops on both sides of the catalytic domain, which may help to determine the binding specificity. The SMB and PDE domains are connected by the first loop (Lα linker region), whereas the PDE and NUC domains are connected by the second loop (Lγ linker region).

The secreted ATX is a constitutively active glycoprotein with a N-terminal signal peptide sequence containing a furin cleavage site. The other ENPPs are transmembrane or anchored proteins. In addition to its pyrophosphatase/phosphodiesterase activities, ATX has lyso phospholipase D (lysoPLD) activity. The N-terminal signal peptide of the ATX precursor is removed first, and then, the remaining part is cleaved by proprotein convertases before the active ATX with lysoPLD activity is released into the extracellular environment, which converts LPC into LPA and choline. The structure of PDE domain has a lipid binding pocket and a nearby tunnel allowing entry of substrates and release of products. The NUC domain has been thought to maintain the rigidity of the PDE domain, and the two N-terminal SMB domains mediate binding of ATX to integrin. This binding brings ATX to the cell membrane, which allows the production of LPA in a location close to its receptors.

The structures of ATX in complex with diverse LPAs show distinct conformations after different acyl chains occupy the binding pocket. LPAs with saturated chains bind in the hydrophobic pocket in a more elongated fashion, whereas LPAs with unsaturated chains have a bent conformation due to the presence of carbon-carbon double bond(s). For LPA, the acyl chain shows a U-shaped conformation in the binding pocket. ATX prefers LPC species with shorter and unsaturated acyl chain as substrates, and the rank order is 14:0 > 16:0 > 18:3 > 18:1 > 18:0 > 14:0 > 16:0 > 18:3 > 18:1 > 18:0. All these show that ATX is able to hydrolyze LPCs with different lengths and saturations of acyl chains to produce the corresponding LPAs.

The cDNA of ATX/ENPP2 was cloned in 1994. After that, its homology with phosphodiesterases was revealed, and the cloning and tissue distribution of the three human and mouse isoforms (α, β and γ) were determined in 2000. Two more isoforms (δ and ε) were identified in 2012. The ATX gene is located on mouse chromosome 15 and on human chromosome 8. The human and mouse ATX gene structures are conserved. The mouse ATX gene spans more than 80 kb and contains at least 27 exons. The three splicing sites in exons 12, 19 and 21 can theoretically result in eight isoforms, which in which five were detected. These isoforms are catalytically active (ATXα-δ) and expressed in different tissues. They are ATXα (ATXαm), ATXβ (ATXαe), ATXγ (PD-Ig) and ATXδ and ATXε, which are the most and second most abundant isoforms, respectively, share similar biochemical characteristics (Figure 2). Houben et al. characterized that a 52-residue polystyrene insertion corresponding to exon 12 in ATXα isoform confers specific binding to heparan sulfate proteoglycans thereby targeting LPA production to the plasma membrane. This is another potential mechanism for localizing ATXα to cell membranes and for LPA production in close proximity to LPA receptors. Exon 12 encodes a 52-amino acid insertion of the mouse ATXα and ATXε.
isoforms (amino acids 324-375), whereas exon 21 encodes an additional 25-amino acid of the murine ATXγ isoform (amino acids 593-617). Novel isoforms ATXδ and ATXξ have a 4-amino acid deletion on exon 19. This complex way of exon arrangement has been maintained through evolution. Human ATX exhibit 93% sequence identity with rodent ATX while all important residues are highly conserved. ATX has a broad profile of tissue expression, with relatively high levels in the blood, brain, kidney, and lymphoid organs. Secretion of ATX leads to high concentration in cerebrospinal fluid and in the endothelial venules of lymphoid tissues. The cellular sources of plasma ATX are incompletely understood. Nevertheless, adipocytes may be a source. ATX is also stored in platelets and released during their activation. Circulating ATX is rapidly taken up by the scavenger receptors of liver sinusoidal endothelial cells, and then degraded by the liver. For the ATX isoforms, high expression levels of ATXγ and ATXξ mRNA were detected in peripheral tissues and the brain, whereas ATXα was shown the lowest expression level in both the central nervous system and peripheral tissues among the three isoforms in human. In mice, ATXδ is widely expressed in the brain and peripheral tissues, and ATXγ and ATXξ showed little variation in their distribution. Human brain and retina showed relatively higher expression level of ATXδ than that of ATXγ and ATXξ, whereas the expression levels of ATXδ and ATXξ in the small intestine and spleen are higher than that in other tissues.

LPA has been quantified in a variety of species, tissues, and fluids, including neural tissue, cerebrospinal fluid, fertilized hen white, seminal fluid, tears, plasma, serum, urine, saliva, and aqueous humor. The formation of LPA species depends on the precursor PLSs, which can vary by acyl chain length and degree of saturation. The term LPA most often refers to 18:1 oleyl-LPA (1-acyl-2-hydroxy-sn-glycero-3-phosphate), as it is the most common one. Other chemical forms of LPA can be observed in various biological systems that have concentrations ranging from low nanomolar to micromolar levels. LPA concentrations in human and rat blood can range from 0.1 μmol/L in plasma and up to 10 μmol/L in serum, which is well over the apparent nanomolar kDa of LPA. The LPA molecules containing 18:2, 20:4, 16:1, 16:0, and 18:1 acyl chains are particularly abundant in plasma. Current methods to detect LPA include indirect enzymatic assays, TLC-GC, LC-MS, and LC-MS/MS.

**LPA RECEPTORS-MEDIATED LPA SIGNALING**

LPA acts as a potent mitogen, which was previously known as “ventricular zone gene-1 (vzg-1)” due its high level in the embryonic neuroproliferative layer of the cerebral cortex. The cloning and functional identification of LPA receptors led to determination of other receptor genes based upon sequence homology. This is particularly true for the “endothelial differentiation gene” (EDG) members that include LPA and sphingosine 1-phosphate receptors. Then, two other LPA receptors, LPA1 and LPA5 (also known as EDG4, and EDG7), were subsequently discovered based on shared homology with LPA.(EDG2). Later on, LPA4 (P2RY9, GPR23) and LPA6 (GPR92) and LPA2 (P2RY5, GPR87) were identified. They share 35% amino acid homology to the purinergic (P2Y) family of GPCRs, as compared to less than 20% homology to LPA1, suggesting that LPA4-6 are more closely related to the P2Y receptors. Here, LPA1-LPA6 are for proteins, and their gene symbols are LPAR1-LPAR6 for human and Lpar1-Lpar6 for non-human.

All LPA receptors signal through at least one of the four heterotrimeric G proteins (Gα12/13, Gαq, and Gα11). They are for proteins, and their gene symbols are for human and gα11 homology to the purinergic (P2Y) family of GPCRs, as compared to less than 20% homology to LPA1, suggesting that LPA4-6 are more closely related to the P2Y receptors. Here, LPA1-LPA6 are for proteins, and their gene symbols are LPAR1-LPAR6 for human and Lpar1-Lpar6 for non-human.

The LPA-coupled G proteins (Gα12/13, Gαq, and Gα11) resulting in downstream signals that produce diverse physiological and pathophysiological effects. Figure 3). Gα12/13-mediated LPA signaling regulates cytoskeletal remodeling, cell migration and invasion through activation of Rho pathway proteins. Rho signals to c-jun N-terminal kinase (JNK) and p38 through Rho-associated kinase (ROCK) and protein kinase N. The LPA-coupled Gα11 protein primarily regulates Ca2+ homeostasis through phospholipase C (PLC), which generates the second messengers IP3 and diacylglycerol (DAG) and Gα11- and Gα13 subunits mediate the activation of phosphatidylinositol 3-kinase (PI3K) which results in the stimulation of the Akt pathway and increase of protein translation after activation of the mammalian target of rapamycin (mTOR) signaling pathway. Activation of PI3K by Gα11 subunits also stimulates the activity of Rac, leading to cell migration and JNK regulation of pro-inflammatory gene expression, and Ras activity, leading to the stimulation of Raf- mitogen-activated protein kinase (MEK)-extracellular signal-regulated kinase (ERK) pathway to promote the expression of genes involved in proliferation and invasion. Gα11, besides PI3K, also stimulates the Ras-Raf-MEK-ERK pathway promoting cell survival and other functions. Gα12/13 can activate adenylyl cyclase and increase cAMP concentration upon LPA stimulation. However, the same enzyme is also inhibited by Gα12/13, showing the complexity of signaling pathways after the activation of LPA receptors.

All six LPA receptors can be stimulated by 1-acyl-LPAs, which show different potencies. LPA1 and LPA4 prefer unsaturated 2-acyl-LPA, while LPA5 likes ether-linked 1-alkyl-LPA species. In addition, lysophosphatidylserine, lysophosphatidylinositol, and lysophosphatidylethanolamine, have been thought to activate these receptors as well. Different LPA molecules may have preference to different subtypes of LPA receptors. Table 1 summarizes PLA receptors expression profiles and their known physiological functions in humans and mice.
LPA

LPA₁ is the first LPA receptor identified based on studies of LPA in the brain[11]. LPA₁ couples to three Gα proteins - Gα₁₂/13, Gα₁₁, and Gαᵢ/₀, which can result in the activation of downstream pathways including Akt, Rho, Ras, and PLC (Figure 3). These pathways mediate many cellular responses initiated by LPA₁ such as neurodevelopment regulation, cell proliferation, differentiation, apoptosis and survival, cell-cell contact through a variety of mechanisms[68,84,103-106]. Dysregulation at glutamatergic synapses was observed in Lpar1⁻/⁻ mice[108]. When the original Lpar1⁻/⁻ mouse line was expanded, a spontaneous variant named "Málaga LPA1" arose. They showed more severe brain defects than the original Lpar1⁻/⁻ line mice did[109]. The loss of LPA₁ in animals seems to modulate the development of several diseases including cancer, obesity, neuropathic pain, fibrosis and male infertility[110].

LPA₂

The amino acid sequence of LPA₂ is about 50% identical to that of LPA₁, and it associates with Gαᵢ/₀, Gα₁₁, and Gα₁₂/13, the same as LPA₁[106] (Figure 3). These G proteins use Ras, PI3K/Rac, PLC/DGA and Rho to mediate their
# Table 1: Expression pattern of lysophosphatidic acid receptors and their known physiological functions in humans and mice

| Name | Information | Previous orphan names | Major expression tissue (high to low level) | Knockout effects in mouse | Biological functions | Ref. |
|------|-------------|-----------------------|---------------------------------------------|---------------------------|----------------------|------|
| LPA3| Human chromosome locus 9q31.3; 41.1 kDa; 364 aa; Identity 97.3% | vsg-1, edg-2, 39.4 kDa; 344 aa | Brain, placenta, urinary bladder, uterus, testis, lung, small intestine, heart, stomach, kidney, spleen, thymus, and skeletal muscle | Perinatal lethality, retarded growth, defective olfaction, reduced body size, craniofacial dysmorphism with blunted snouts, and increased apoptosis in sciatic nerve Schwann cells | Neurodevelopment regulation, cell proliferation, differentiation, apoptosis and survival, cell-cell contact through serum-response element activation, cell migration and cytoskeletal organization, Ca2+ homeostasis, cAMP-regulated cellular processes and adenylyl cyclase inhibition | Yang et al.[116], 2014; Archbold et al.[112], 2008; Chou et al.[117], 2010; Anliker et al.[118], 2013; Sakai et al.[119], 1999; An et al.[120], 1998; Contos et al.[121], 2000; Contos et al.[122], 2000; Fukushima et al.[123], 2001. |
| LPA3| Human chromosome 19p13.11; 39.1 kDa; 351 aa; Identity 85.3% | edg-4, IpA2 | Leukocytes, testis, prostate, spleen, thymus and pancreas. | Normal | Cell migration, viable and healthy, nervous system development and immune system regulation. | Yang et al.[124], 2014; An et al.[125], 1998; Contos et al.[126], 2000b; Archbold et al.[127], 2014; Ohuchi et al.[128], 2008; Chou et al.[129], 2010; Valentine et al.[130], 2008; Xu et al.[131], 2004; Lai et al.[132], 2005; Contos et al.[133], 2002; Chou et al.[134], 2008; Yang et al.[135], 2014; Bandoh et al.[136], 1999; Ima et al.[137], 2000; Contos et al.[138], 2002a; Zhao et al.[139], 2015; Ye et al.[140], 2010; Hama et al.[141], 2010; Lai et al.[142], 2012. |
| LPA3| Human chromosomal locus 1p22.3; 40.1 kDa; 353 aa; Identity 90.2% | edg-7, IpA3 | Heart, testis, prostate, pancreas, lung, ovary, and brain. | Delayed embryonic implantation, embryonic crowding, and reduced litter size for female null mutants. | Male and female reproductive physiology, inflammation, cell Ca2+ homeostasis and cAMP regulation, vertebrate left-right patterning during embryogenesis. | Yang et al.[143], 2014; Ohuchi et al.[144], 2008; Chou et al.[145], 2010; Liu et al.[146], 2010; Manswell et al.[147], 2010; Liu et al.[148], 2009; Sumida et al.[149], 2010; Yanagida et al.[150]; Lee et al.[151], 2007; Rhee et al.[152], 2006; Lee et al.[153], 2008. |
| LPA3| Human chromosome 3q21.1; 41.9 kDa; 370 aa; Identity 98.4% | P2Y1/GPR23 | Ovaries, thymus, pancreas, brain, heart, small intestine, testis, prostate, colon, and spleen. | Inhibition of its differentiation into osteoblasts in human mesenchymal stem cell line; For mouse: increased trabecular bone volume, number, and thickness; pericardial effusions, severe edema and hemorrhage, abnormally dilated blood and lymphatic vessels and lymph sacs, and impaired pericyte recruitment. | ROCK-dependent cell aggregation and N-cadherin-dependent cell adhesion, cAMP accumulation, differentiation of immortalized hippocampal progenitor cells, negatively cell motility regulation and osteogenesis. | Yang et al.[154], 2014; Ohuchi et al.[155], 2008; Chou et al.[156], 2010; Liu et al.[157], 2010; Manswell et al.[158], 2010; Liu et al.[159], 2009; Sumida et al.[160], 2010; Yanagida et al.[161]; Lee et al.[162], 2007; Rhee et al.[163], 2006; Lee et al.[164], 2008. |
| LPA3| Human chromosome 12p13.31; 41.3 kDa; 372 aa; Identity 79.0% | GPR92 | Spleen, heart, small intestine, placenta, colon, and liver. | Neurite retraction, stress fiber formation, receptor internalization, water absorption, Ca2+ mobilization and cAMP accumulation, LPA-induced release of chemokine ligand 4 in mast cells. | | Yang et al.[165], 2014; Lee et al.[166], 2006; Lee et al.[167], 2015; Anisten et al.[168], 2008; Lundequist et al.[169], 2011; Araki et al.[170], 2014; Lin et al.[171], 2010; Yanagida et al.[172], 2013. |
down-stream signals, which may regulate cell survival and migration[107]. LPA3 regulates cell survival and cell migration in the development of nervous system and functions of immune system[68,90,106,110,111]. The focal adhesion molecule thyroid receptor-interacting protein 6[102,112] and several PDZ-domain and zinc finger proteins[114] interact with LPA3. The PDZ-binding domain of LPA3 regulates Na+/H+ exchanger regulatory factor 2 activity, and activates PLC-3 and Akt/ERK signaling pathways. These pathways stimulate cell migration, enhance survival, and alter gene expression, accounting for the functions attributed to LPA3. Lpar2−/− mice are viable and healthy, while those null for both Lpar1 and Lpar2 show features essentially consistent with those of Lpar1−/−[145]. These data suggest functional redundancy of LPA3 with LPA4.

**LPA4**

LPA4/LPAR4 was cloned based upon homology to already identified LPA receptor genes using degenerated primers in a PCR-based cloning strategy[83,116]. LPA4 couples with Gq/11 and Go12, to mediate adenylyl cyclase inhibition, PLC activation and Ca2+ mobilization, and Ras activation[109] (Figure 3). LPA4 prefers 2-acyl-LPAs containing unsaturated fatty acids[89]. It mediates the activation of a series of physiological processes such as male and female reproductive physiology, inflammation, cell Ca2+ homeostasis and cAMP regulation[107,117-119]. LPA4 appears to determine vertebrate left-right patterning during embryogenesis as downregulation of Lpar3 or inhibition of LPA4 activity disrupted patterning process in zebrafish[120]. Lpar3−/− mice are viable with no reported neural defects, even though LPA4 is found in the frontal cortex, hippocampus, and amygdala[83,116]. On the other hand, female Lpar3−/− mice have a delayed embryo implantation, and reduced litter size[117].

**LPA5**

The first so-called non-EDG LPA receptor was identified in 2003, and named as LPA5. It shares homology (approximately 20%) with LPA1-3, and it is more close to the P2Y receptor family[86]. LPA5 was identified by screening orphan receptors using calcium mobilization as a readout for ligand-induced signals. LPA4 couples with Gq/11, Go12, Gs and Go14, and activates Rho/ROCK to induce neurite retraction and stress fiber formation[71,97] (Figure 3). It induces ROCK-dependent cell aggregation and N-cadherin-dependent cell adhesion[71]. LPA5 is only LPA receptor that activates Gq/11 to induce cAMP level[97].

The activation of LPA4 was thought to regulate the differentiation of immortalized hippocampal progenitor cells[121]. In addition, the activation of LPA4 could inhibit LPA4-induced cell migration, but LPA4 exposure increased lamellipodia formation and transwell movement of LPA4 null cells, indicating an increased sensitivity[122].

It shows the ability of LPA5 to negatively regulate cell motility and indicates that differential effects may be achieved by simultaneously expressing multiple LPA receptors. LPAR4-deficient human mesenchymal stem cells lost ability to differentiate into osteoblasts[123]. While adult Lpar4−/− mice appear grossly normal[122], they exhibit increased trabecular bone volume, number, and thickness[124,125]. LPA4 pathway seems to inhibit osteogenesis. Lpar4−/− mice had reduction of prenatal survival rate during embryo development, which is accompanied by changes such as pericardial effusions, severe edema and hemorrhage[126].

**LPA6**

LPA6, the fifth LPA receptor, was identified in 2006[87,127]. It shares about 35% homology with LPAR4, and 22% homology with LPAR1-3[87]. LPA6 couples with Gq/11, Gq/12/13, and Gq/11, which mediate neurite retraction, stress fiber formation, and receptor internalization in LPA4-expressing cell lines[87] (Figure 3). It also activates Gq/11 to increase intracellular calcium mobilization, and cAMP accumulation via a non-Gs mechanism, suggesting the involvement of other G-proteins[97,127]. LPA6 signaling may also affect intestinal water absorption[128]. This is achieved through the LPA4-induced recruitment of Na+/H+ exchanger 3 to the microvilli mediated by the interaction between LPA6 and Na+/H+ exchanger regulatory factor 2. Additionally, LPA6 is the main LPA receptor responsible for LPA4-induced release of chemokine ligand 4 in mast cells[129].

Interestingly, LPA6 in B16 melanoma cells, prefers alkyl-LPA (18:1) to acyl-LPA (18:1)[87]. Lpar5−/− null mice exhibit reduced lung metastasis by melanoma cells compared with wild type ones[130].

**LPA7**

The most recently identified LPA receptor is LPA7. It was first isolated from a chicken T cell library and named receptor 6H1 in 1993[131], and then, renamed to P2Y5 because of sequence homology with P2Y receptors in
from adipose tissue, ATX may promote preadipocyte expression more ATX than preadipocytes. When secreted weight kinase Ras-Raf-MEK-ERK pathway, and of the focal adhesion growth of 3T3F442A cells signaling in fat storage of 3T3F442A preadipocytes, indicating the role of LPA differentiation. LPA was found to induce proliferation accumulation in adipocytes. Hyperplasia results in hyperplasia. Hypertrophy results in excessive TAG Obesity is associated with adipocyte hypertrophy and LPA receptor signaling regulates adipogenesis cells, including an increased ratio of adipocyte precursor factors modulate the propensity to accumulate fat in factors contribute to the obesity development. Many problems correlate with premature death, cardiovascular disease, of cancer. The long-term effects of being overweight including diabetes, heart disease, stroke, and some types were obese in 2011-2014. Obesity is associated with the development of chronic metabolic diseases including diabetes, heart disease, stroke, and some types of cancer. The long-term effects of being overweight correlate with premature death, cardiovascular disease, metabolic morbidities, and asthma, among other problems. Both environmental factors and genetic factors contribute to the obesity development. Many factors modulate the propensity to accumulate fat in cells, including an increased ratio of adipocyte precursor cells to differentiated adipocytes.

**LPA RECEPTOR SIGNALING IN OBESITY AND INSULIN RESISTANCE**

Recently, obesity has become major public health concern, particularly in the United States. According to 2015 Center of Disease Control and Prevention estimates, more than one-third of adults (34.9% or 78.6 million) and 17% of youth in the United States were obese in 2011-2014. Obesity is associated with the development of chronic metabolic diseases including diabetes, heart disease, stroke, and some types of cancer. The long-term effects of being overweight correlate with premature death, cardiovascular disease, metabolic morbidities, and asthma, among other problems. Both environmental factors and genetic factors contribute to the obesity development. Many factors modulate the propensity to accumulate fat in cells, including an increased ratio of adipocyte precursor cells to differentiated adipocytes.

**LPA receptor signaling regulates adipogenesis**

Obesity is associated with adipocyte hypertrophy and hyperplasia. Hypertrophy results in excessive TAG accumulation in adipocytes. Hyperplasia results in recruitment of new adipocytes via proliferation and differentiation. LPA was found to induce proliferation of 3T3F442A preadipocytes, indicating the role of LPA signaling in fat storage. LPA stimulation increases the growth of 3T3F442A cells via LPA, which activates the Ras-Raf-MEK-ERK pathway, and of the focal adhesion kinase.

It has been reported that Lpar1/- mice exhibited greater adiposity than the control mice without alteration of feeding behavior, despite of lowered body weight. Interestingly, Lpar1/- mice were resistant to diet-induced obesity that may result at least in part from alterations in leptin production. Mature adipocytes express more ATX than preadipocytes. When secreted from adipose tissue, ATX may promote preadipocyte proliferation. Its expression was up-regulated during adipocyte differentiation, and in db/db mice.

The serum levels of LPC, the precursors of LPA, increases gradually in rabbits fed a high-cholesterol diet for 12 wk. The levels of individual LPAs formed after the incubation of serum for 24 h elevated with the increase of the length of time that rabbits were fed a high cholesterol diet. These studies indicate that feeding of a high-fat diet can cause an increase in the circulating level of LPA. Preadipocytes mainly express LPA, and the mRNA level of Lpar1 expression in preadipocytes is higher than that in mature 3T3-L1 adipocytes. However, in human adipose tissue, obesity does not influence on LPAR1 expression. This discrepancy of LPA expression levels between human and mouse adipose tissues suggest that obesity promotes LPA synthesis rather than activation in adipose tissue.

The LPA-induced proliferation of preadipocytes has been thought to be mediated through LPA and the activation of the Ras-Raf-MEK-ERK pathway. LPA inhibits differentiation of white and brown preadipocyte cell lines, which include porcine preadipocyte cell line; mouse preadipocyte cell line, 3T3-L1 and 3T3F442A; and human Simpson-Golabi-Behmel Syndrome preadipocyte cells. This inhibition is mediated by LPA via the Rho-ROCK pathway. All these results in a down-regulation of PPARγ, and impaired responses of PPARγ-targeted genes to its ligands, which leads to reduced TAG accumulation, and expression levels of adipogenic genes.

The activation of Rho-ROCK pathway delayed the activation of the Wnt-signaling pathway, which has been partially attributed to the inhibited PPARγ expression and adipogenesis. When mice with the adipocyte-specific knockout of ATX gene (FATX-KO) were fed a high-fat diet, they had more fat mass and larger adipocyte size, but not adipocyte number, than the control mice did in the absence of any change of food intake. The deletion of ATX in mice appeared to lead sensitivity to diet-induced obesity, which might be due to elevated expression levels of PPARγ and its downstream adipogenic genes in subcutaneous white adipose tissue. Interestingly, those knockout mice had improved glucose tolerance and less systemic insulin resistance than the control mice fed the same diet. LPA stimulation seems to have anti-adipogenic effect in white adipocytes, and in brown preadipocytes. Aforementioned experiments seem to indicate that ATX-LPA receptor signaling pathway may inhibit the development of adipose tissue.

On the other hand, others reported that ATX promotes preadipocytes proliferation and differentiation into adipocytes, thereby promoting adipocyte hyperplasia and obesity. It was showed that deletion of ATX results in smaller body weight gain, smaller fat pad weights and adipocyte numbers, less insulin resistance and glucose tolerance in heterozygous Enpp2/- mice and adipocyte-specific FATX-KO mice fed a high-fat diet than their littermates controls. Moreover, the FATX-KO improved brown adipose tissue function,
In adipose tissue, LPA also regulates glucose metabolism in obese and insulin-resistant subjects and mice. Increased LPA, as the precursor of lysophosphatidic acid (LPA), are reduced in obese and type 2 diabetic mice, suggesting a negative effect of LPA on glucose homeostasis in them. LPA was found to enhance glucose uptake in a dosedependent manner in both GLUT4-myc L6 myotubes and 3T3-L1 adipocytes, a process that was attributed to the increase of GLUT4 translocation in a P13K dependent manner. Moreover, the effect of LPA on glucose uptake was completely inhibited by pretreating cells with P13K receptor antagonist Ki16425 and Gi inhibitor pertussis toxin. LPA significantly lowered blood glucose levels in normal mice and streptozotocin-induced diabetic mice, suggesting the promotion of glucose usage, but not stimulation of insulin secretion.

The elevation of ATX expression in adipocytes of db/db mice occurred simultaneously with the development of hyperglycemia, and only 3 wk after the emergence of hyperinsulinemia in them. ATX expression was up-regulated by treatment with TNFα, and down-regulated by rosiglitazone in 3T3F442A adipocytes. The upregulation of ATX expression in adipocytes of db/db mice seems to be associated with the emergence of hyperglycemia rather than fat accumulation or hyperinsulinemia.

The plasma levels of LPC, as the precursor of LPA, are reduced in obese and type 2 diabetic mice, suggesting that it may regulate blood glucose level. This reduction may contribute to the impairment of glucose homeostasis. Interestingly, adipocyte specific ATX knockout mice fed with a high-fat diet showed greater adiposity and better tolerance to glucose challenge than control mice, suggesting a negative effect of LPA on glucose homeostasis. Similarly, LPA production appears to impair glucose disposal probably through a reduction in the circulating ATX levels correlated negatively with body mass index, and mRNA levels of ATX were reduced in visceral fat, and the latter fat pad in obese subjects.

In mice, ATX is highly expressed in visceral white adipose tissue and brown adipose tissue metabolism. Transgenic mice expressing the human ATX/ENPP2 gene under the control of α1 antitrypsin gene promoter became sensitive to diet-induced obesity due to reduced expression of brown adipose tissue-related genes in peripheral white adipose tissue and accumulated significantly more fat without any change of locomotor activities, thermogenic profiles, and systemic metabolism. In human, ATX expression is higher in subcutaneous than in visceral fat, and is downregulated in adipose tissue hypertrophy. In human ATX expression is higher in subcutaneous than in visceral fat, and the latter fat pad in obese subjects has higher ATX expression level than that in non-obese subjects, which is correlated with leptin expression. The circulating ATX levels correlated negatively with body mass index, and mRNA levels of ATX were reduced in subcutaneous fat from obese subjects. Moreover, ATX expression in adipose tissues may be negatively regulated by LPA through a feedback regulatory mechanism, which may involves inflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin-1β.

**Figure 4** Autotaxin-lysophosphatidic acid signaling axis regulates adipose tissue development and glucose homeostasis in obesity. In adipose tissue, especially in mature adipocytes, the elevated expression of ATX leads to production of LPA and then induced proliferation of preadipocytes via LPA through Ras-Raf-MEK-ERK pathway. On the other hand, LPA inhibits differentiation of white and brown preadipocytes, which is mediated by LPA via the Rho-ROCK pathway. Short-term insulin treatment increases ATX secretion in adipocytes via P13K/Akt-mTOR pathway, whereas long-term insulin treatment reduces ATX activity. LPA produced by ATX in obesity has a tonic inhibitory effect on glucose homeostasis through inhibition of insulin secretion in isolated pancreas islets, increase of glucose transport in myocyte and adipocytes via GLUT4 translocation in a P13K dependent manner, and elevation of glycogenolysis in hepatocytes. ROCK: Rho-associated kinase; P13K: Phosphatidylinositol 3-kinase; MEK: Mitogen-activated protein kinase; ERK: Extracellular signal-regulated kinase; Akt: Protein kinase B; mTOR: Mammalian target of rapamycin; GLUT4: Glucose transporter type 4.
of plasma insulin as pharmacological inhibition of LPAR₁α activation improves glucose homeostasis in obese and prediabetic mice [171]. Another possibility is that the progression of diabetes affects ATX expression in adipose tissue [162]. It has been shown that treatments with high concentrations of glucose and insulin led to ATX secretion in adipocytes. Short-term insulin treatment increased ATX activity, whereas long-term insulin treatment reduced the levels of ATX mRNA and protein, and its activity [172].

In humans, ATX expression in adipose tissue significantly increased in diabetes patients in contrast with obese-only subjects [156,166]. Its expression in subcutaneous fat is higher than that in visceral fat. Nevertheless, ATX in visceral, but not subcutaneous, fat of obese subjects is higher than that in non-obese patients [155]. Interestingly, the circulating ATX levels in the blood were reduced in obese subjects [182]. The females have higher blood ATX level than males [173].

The variations of ATX expression were correlated with some clinical parameters. In obese patients, visceral fat ATX was positively correlated with diastolic arterial blood pressure, plasma leptin level, and expression levels of inducible nitric oxide synthase and apelin receptor [155]. In older and obese humans, plasma ATX correlated with fasting glucose, fasting insulin, and glucose level 2 h after an oral glucose tolerance test, and body mass index [173]. LPA produced by ATX in obesity has a tonic inhibitory effect on glucose homeostasis through inhibition of insulin secretion in isolated pancreas islets, increase of glucose transport in myocyte and adipocytes, and elevation of glycogenolysis in hepatocytes [164]. LPA was reported to activate glycogenolysis in hepatocytes in vitro [174], suggesting that LPA’s effects on glucose homeostasis may be mediated by the liver. All these indicate that LPA production via ATX and its receptors activation may impact glucose homeostasis (Figure 4).

### POSSIBLE ROLE OF LPA SIGNALING IN LIVER FIBROSIS

The liver plays a critical role in the control of glucose and lipid homeostasis. The disturbance of this homeostasis may lead to development of metabolic diseases such as type 2 diabetes [179] and nonalcoholic fatty liver disease (NAFLD) [176]. Liver fibrosis is a process that leads to the alteration of the hepatic architecture marked by the accumulation of proteins such as collagen in extracellular matrix. This is generally associated with the development of various liver diseases such as NAFLD and hepatitis. If left untreated, the further development of these diseases and liver fibrosis will lead to cirrhosis, and liver failure, which needs liver transplantation for the treatment. Factors causing damages of hepatocytes result in activation of hepatic stellate cells (HSCs) and production of pro-inflammatory and pro-fibrotic factors, which will stimulate formation of accumulation of proteins in extracellular matrix [177].

The injuries caused by nutritional and environmental factors alter liver structures and functions, which may lead to the liver fibrosis [178]. The hepatic matrix is remodeled by the inflammatory responses after liver injury. Upon the stimulation, the generation of the liver matrix such as collagen, elastin, hyaluronan, proteoglycans and fibronectin is elevated, which is followed by remodeling processes. All these are associated with the activation of HSCs, and the change of local architecture and the reduction of liver functions. Excessive production and accumulation of extracellular matrix in the liver results in fibrosis, which can lead to liver cirrhosis [178]. In addition to HSCs, other cells responsible for the fibrosis include fibrocytes from hematopoietic stem cells, portal fibroblasts, bone marrow derived mesenchymal cells, epithelial-mesenchymal transition and endothelial to mesenchymal transition [178].

The excessive accumulation of lipids and alterations of their metabolism have been used to explain the etiology of type 2 diabetes, which is associated with profound changes of hepatic gene expression [175,179]. This alteration of hepatic lipid metabolism may cause the development of fibrosis. For example, the elevation of lipid peroxidation in zone 3 hepatocytes has been suggested with the development of fibrosis [180]. On the other hand, changes of fatty acid compositions in plasma phospholipids have been observed in subjects with fibrosis [181,182]. All these show that the alterations of plasma phospholipids in patients with metabolic diseases may play a role in the development of fibrosis.

It has been shown that serum ATX activity and LPA levels increase with the development of liver fibrosis in patients with chronic hepatitis C [74,183-185], and with cholestasis and pruritus [186,187]. The association of elevated plasma ATX level with chronic liver disease (CLD) in patients suggests a shorter overall survival in a 10-year follow-up study [185]. Moreover, the increased expression level of hepatic ATX mRNA was found in the majority of publically available CLD and hepatocellular carcinoma (HCC) microarray data sets, suggesting an association of ATX with liver pathophysiology [185]. ATX and LPA levels increased in the plasma of patients with hepatitis C virus (HCV) infection, and positively associate with liver fibrosis stages [183,184,188,189]. HCV infection may stabilize the activity of hypoxia inducible factor in a PI3K dependent manner, which may increase ATX expression, and in turn induce liver fibrosis [190].

It has been shown that serum ATX level correlated with fibrosis grade, and is useful as its marker in liver fibrosis [191]. The higher expression level of LPA2 mRNA has been associated with the poorer differentiation of HCC cells, and a higher LPA6 mRNA level is associated with microvascular invasion of HCC. The high expression levels of LPA2 and LPA6 mRNA in HCC predict a high potential for malignancy. The elevated levels of LPA6 and LPA6 mRNA in conjunction with plasma ATX predict a higher rate of recurrence after surgical removal of the tumors [192]. In addition, the plasma level of ATX
has been considered as a potential pathogenic factor and/or biomarker for nonalcoholic fatty liver disease in nondiabetic and obese women\(^{193}\). Moreover, the plasma ATX levels correlated with prognosis of cirrhosis (Child-Pugh score), showing the link of ATX and the severity of cirrhosis in patients with CLD\(^ {194} \). In rats, plasma LPA level and serum ATX activity were increased in liver injury and were correlated with severity of the damage; the former in relation to the extent of fibrosis, and the latter in relation to the extent of hepatocyte damage\(^ {186,195} \). In mice, different hepatotoxic stimuli linked with the development of different forms of CLD were shown to stimulate hepatocyte ATX expression, leading to increased LPA production, HSCs activation, and signals for fibrosis development\(^ {186} \).

LPA was first shown to stimulate rat HSCs proliferation through MAP kinase activation in 1998\(^ {196} \). Then, LPA was shown to enhance HSCs contractility through modulation of cellular morphology and attachment to extracellular matrices via Rho-kinase\(^ {197,198} \). LPA also inhibits the apoptosis of those cells through Rho/Rho kinase activation\(^ {199} \), suggesting its involvement in the pathogenesis of liver fibrosis. Moreover, LPA was shown to induce nuclear translocation of inducible nitric-oxide synthase in hepatocytes\(^ {200} \). These findings demonstrate the possible involvement of LPA in the development of liver fibrosis.

**CONCLUSION**

LPA is a highly bioactive lipid mediator with a number of cellular sources and exerts its actions through a family of receptors coupling with GPCRs in various cell types. Here, we have discussed recent advances in pathways for extracellular and intracellular production of LPA, the functions as well as structural and biochemical properties of ATX and LPA receptors. For the past 20-30 years, the cloning and identification of proteins mediating LPA production and signal transduction pathways open a new field for us to understand relevance of these proteins in physiology and disease development. The association of LPA production and signal pathways with chronic metabolic diseases has been gradually realized. We have highlighted the roles of LPA signaling pathways in the obesity, insulin resistance and liver fibrosis.

The realization of the importance of LPA-mediated functions leads to more open questions begging for answers. (1) The regulations of enzymes involved in LPA synthesis and degradation pathways remain to be further investigated. Whether intracellularly produced LPA can cross the plasma membrane into the extracellular compartment is currently unclear. Additional enzymes or pathways for the production of LPA are still worth exploring. For example, phosphatidylglycerol was shown to be converted to LPA under the catalytic action of GPAT as reported\(^ {201} \). (2) For ATX, an important player for the extracellular LPA production, how its activity is regulated, and how the newly produced LPA is released remain to be addressed. Future analysis will undoubtedly shed some light on these. (3) There are many factors contributing to the pathophysiology of obesity and metabolic diseases. Therefore, the precise role of LPA signaling pathways in these diseases remains to be investigated further. In addition, mechanisms by which the LPA and its receptor signaling pathways in the differentiation of both white and brown adipocytes remain to be clarified. This may help for the control of lipid metabolism. (4) LPA seems to have a negative effect on glucose homeostasis in obesity. This was observed only in obese patients, but not in non-obese subjects. So future human studies should focus on more healthy subjects and compare with those parameters of obese patients. (5) LPA appears to inhibit insulin secretion. Whether this inhibitory effect is due to a direct action of LPA on pancreas islets or a possible regulation of liver glycogen mobilization and/or muscle glucose oxidation remains to be clarified. And (6) It was shown that the plasma LPA level and serum ATX activity both were increased in association with liver fibrosis. The underlying mechanism remains to be determined.

Taken together, the LPA signaling pathways contain multiple points that potentially involve in the development of obesity, liver fibrosis and related pathologies. The development of novel pharmacological modulators targeting intervention points may open new research fields and provide potential medicinal therapies to reduce human suffering. The prospects are bright for expanding insights and contributions in LPA biology.

**ACKNOWLEDGMENTS**

The authors wish to thank Dr. Elizabeth (Betsy) Anderson Steeves (Department of Nutrition at the University of Tennessee, Knoxville, TN, United States) for her critical reading and editing of this manuscript. In addition, we also would like to thank Dr. Jianan Qi (Southeast University, China) for figures editorial assistance.

**REFERENCES**

1. van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. *Nat Rev Mol Cell Biol* 2008; 9: 112-124 [PMID: 18216768 DOI: 10.1038/nrm2330]
2. Vogt W. The chemical nature of Darmstoff. *J Physiol* 1957; 137: 154-167 [PMID: 13439591]
3. Vogt W. Pharmacologically active lipidsoluble acids of natural occurrence. *Nature* 1957; 179: 300-304; passim [PMID: 13407704]
4. Vogt W. [Not Available]. *Naunyn Schmiedebergs Arch Exp Pathol Pharmakol* 1949; 206: 1-11 [PMID: 18115712]
5. Vogt W. [Properties and preparation of Darmstoff]. *Naunyn Schmiedebergs Arch Exp Pathol Pharmakol* 1955; 227: 224-233 [PMID: 13309417]
6. Vogt W. Pharmacologically active acidic phospholipids and glycolipids. *Biochem Pharmacol* 1963; 12: 415-420 [PMID: 13997687]
7. Sen S, Smeyh RR, Bumpus FM. Antihipertensive effect of an isolated phospholipid. *Am J Physiol* 1968; 214: 337-341 [PMID: 4295739 DOI: 10.1152/ajplegacy.1968.214.2.337]
8. Tokumura A, Fukuzawa K, Tsukatani H. Effects of synthetic and natural lysophosphatidic acids on the arterial blood pressure of different animal species. *Lipids* 1978; 13: 572-574 [PMID: 703553]
Platelet interaction with bioactive lipids formed by 2003;1999; Silletta MG, Spanò S, Turacchio G, Cericola C, Kaffe E, Aidinis V, Kokotos G. Autotaxin, a 2004; Zangl KJ, Essler M, Bauer M, Brandl R, Corrinth C, Haldar D. Regulation of mitochondrial and 2004; Hla T, Lynch KR, Spiegel S, Moolenaar WH. Lee DP. Enzymes of triacylglycerol synthesis 2004; Ferry G. Autotaxin. O'Sullivan C, Sheridan GK. Lysophosphatidic acid and 2004; Zebisch M, Sträter N. Cellular function and 2001;135: 1071-1083 [PMID: 8922387]

Chun J, Hla T, Lynch KR, Spiegel S, Moolenaar WH. International Union of Basic and Clinical Pharmacology, LXVIII. Lysophospholipid receptor nomenclature. Pharmacol Rev 2010; 62: 579-587 [PMID: 21079037 DOI: 10.1124/pr.100.003111]

Tominaga K, Majima E, Kariya Y, Tominaga K, Kogure K, Yasuda K, Fukuzawa K. Identification of human plasma lysophospholipase D, a lysophosphatic acid-producing enzyme, as autotaxin, a multifunctional phosphodiesterase. J Biol Chem 2002; 277: 39436-39442 [PMID: 12176993 DOI: 10.1074/jbc.M205623200]

Aikawa S, Hashimoto T, Kano K, Aoki J. Lysophosphatic acid as a lipid mediator with multiple biological actions. J Biochem 2015; 157: 81-89 [PMID: 25500504 DOI: 10.1093/jb/mvu077]

Rancoule C, Dusaulcy R, Tréguer K, Grés S, Attané C, Saulnier-Blache JS. Involvement of autotaxin/lysosphosphatic acid signaling in obesity and impaired glucose homeostasis. Biochimie 2014; 96: 140-143 [PMID: 23639740 DOI: 10.1016/j.biochi.2013.04.010]

Barbayianni E, Kaffe E, Aidinis V, Kokotos G, Aikawa S, Hashimoto T, Kano K, Aoki J. Lysophosphatic acid as an intracellular receptor for lysophosphatidic acid and lysophosphatidylcholine formation. J Biol Chem 2001; 276: 70743-70753 [PMID: 11476603 DOI: 10.1074/jbc.M100100200]

Kai M, Wada I, Imai S, Sakane F, Kanoh H. Identification and cDNA cloning of 35-kDa lysophosphatic acid phosphatase (type 2) bound to plasma membranes. Polymerase chain reaction amplification of mouse H2O2-inducible hic53 clone yielded the cDNA encoding lysophosphatic acid phosphatase. J Biol Chem 1996; 271: 18931-18938 [PMID: 8702556]

Leung DW. Tompkins CK, White T. Molecular cloning of two alternatively spliced forms of human lysophosphatic acid phosphatase cDNAs that are differentially expressed in normal and tumor cells. DNA Cell Biol 1998; 17: 377-385 [PMID: 9570154 DOI: 10.1089/ dna.1998.17.377]

Roberts R, Sciorra VA, Morris AJ. Human type 2 lysophosphatic acid phosphohydrolases. Substrate specificity of the type 2a, 2b, and 2c enzymes and cell surface activity of the 2a isoform. J Biol Chem 1998; 273: 22059-222067 [PMID: 9705349]

Aguado B, Campbell RD. Characterization of a human lysophosphatic acid acyltransferase that is encoded by a gene located in the class III region of the human major histocompatibility complex. J Biol Chem 1998; 273: 4096-4105 [PMID: 9461603]

Modregger J, Schmidt AA, Ritter B, Huttner WB, Plomann M. Characterization of Endophilin B1b, a brain-specific membrane-associated lysophosphatic acid acyltransferase with properties distinct from endophilin A1. J Biol Chem 2003; 278: 4166-4147 [PMID: 12456676]

Weigt R, Sillettia MG, Spanó S, Turacchio G, Cericola C, Colanzi A, Senatore S, Mancini R, Polischuk EV, Salmona M, Facchiano F, Burger KN, Mironov A, Luini A, Corda D. CtBP/BARS induces fission of Golgi membranes by acylating lysophosphatic acid. Nature 1999; 402: 429-433 [PMID: 10586885 DOI: 10.1038/46587]

Wang A, Dennis EA. Mammalian lysophospholipases. Biochim Biophys Acta 1999a; 1439: 1-16 [PMID: 1039961]

Stefan C, Jansen S, Bollen M. NPP-type ectophosphodiesterases: unity in diversity. Trends Biochem Sci 2005; 30: 542-550 [PMID: 16125936]

Zimmermann H, Zebisch M, Sträter N. Cellular function and molecular structure of ecto-nucleotidases. Purinergic Signal 2012; 8: 437-502 [PMID: 2681110 DOI: 10.1007/s11302-012-9309-4]

Boutin JA, Ferry G. Autotaxin. Cell Mol Life Sci 2009; 66: 3009-3021 [PMID: 19506801 DOI: 10.1007/s00018-009-0556-9]

Nakanaga K, Hama K, Aoki J. Autotaxin—an LPA producing enzyme with diverse functions. J Biochem 2010; 148: 13-24 [PMID: 20495010 DOI: 10.1093/jb/mvq052]
Identification and biochemical characterization of a novel autotaxin isoform, ATXδ, with a four-amino acid deletion. J Biochem 2012; 151: 89-97 [PMID: 21994952 DOI: 10.1093/jb/mvr112]

Bächner D, Ahrens M, Betat N, Schröder D, Gross G. Developmental expression analysis of murine autotaxin (ATX). Mech Dev 1999; 84: 121-125 [PMID: 10473125]

Nimiphipong H, Holick MF. Vitamin D status and sun exposure in southeast Asia. Dermatoendocrinol 2013; 5: 34-37 [PMID: 24499404 DOI: 10.4161/derm.24054]

Savaskan NE, Rocha L, Kotter MR, Baer A, Lubec G, van Meeteren LA, Kishi Y, Aoki J, Moolenaar WH, Nitsch R, Bräuer AU. Autotaxin (NP-2) in the brain: cell type-specific expression and regulation during development and after neurotrauma. Cell Mol Life Sci 2007; 64: 230-243 [PMID: 17192809]

Brock K, Cant R, Clemson L, Mason RS, Fraser DR. Effects of diet and exercise on plasma vitamin D (25(OH)D) levels in Vietnamese immigrant elderly in Sydney, Australia. J Steroid Biochem Mol Biol 2007; 103: 786-792 [PMID: 17215122 DOI: 10.1016/j.jsbmb.2006.12.048]

Nakasaki T, Tanaka T, Okudaira S, Hiroswa M, Umemoto E, Otani K, Jin S, Bai Z, Hayasaki H, Fukui Y, Aozasa K, Fujita N, Tsuruo T, Ozo no K, Aoki J, Miyasaki M. Involvement of the lysophosphaticidic acid-generating enzyme autotaxin in lymphocyte-endothelial cell interactions. Am J Pathol 2008; 173: 1566-1576 [PMID: 18813830 DOI: 10.1038/ajpath.2008.71155]

Bai Z, Cai L, Umemoto E, Takeda A, Tohya K, Komai Y, Veeraveedu PT, Hata E, Sugira Y, Kubo A, Sue matsu M, Hayasaki H, Okudaira S, Aoki J, Tanaka T, Albers HM, Ovaa H, Miyasaki M. Constitutive lymphocyte transmigration across the basal lamina of high endothelial venules is regulated by the autotaxin/lysophosphaticidic acid axis. J Immunol 2013; 189: 2036-2048 [PMID: 23365076 DOI: 10.4049/jimmunol.1202025]

Dusau rély A, Rancoule C, Grès S, Waneqcé E, Colom A, Guigné C, van Meeteren LA, Moolenaar WH, Valet P, Saulnier-Blache JS. Adi pose-specific disruption of autotaxin enhances nutritional fattening and reduces plasma lysophosphaticidic acid. J Lipid Res 2011; 52: 1247-1255 [PMID: 21421848 DOI: 10.1194/jlr.M014985]
Yang Fe et al. LPA in the regulation of adipocyte functions and liver fibrosis

signaling and biology. *Ann Rev Biochem* 2004; 73: 321-354 [PMID: 15189145]

106 Contos J, Ishii I, Chun J. Lysophosphatidic acid receptors. *Mol Pharmacol* 2000; 58: 1188-1196 [PMID: 11093753]

107 Contos J, Fukushima N, Weisgerber JA, Kaushal D, Chun J. Requirement for the LPA1 lysophosphatidic acid receptor gene in normal suckling behavior. *Proc Natl Acad Sci USA* 2000; 97: 13384-13389 [PMID: 11087877]

108 Musáli Z, Di Dani E, Maycox P, Racagni G, Polopi M. Abnormalities in β1-βCaMKII and related mechanisms suggest synaptic dysfunction in hippocampus of LPA1 receptor knockout mice. *Int J Neuropharmacol* 2011; 14: 941-953 [PMID: 21994290 DOI: 10.1177/1471145610392240]

109 Estivill-Torruñ G, Llebrez-Zayas P, Matias-Rico E, Santin L, Pedraza C, De Diego I, Del Arco I, Fernández-Llebrez P, Chun J, De Fonseca FR. Absence of LPA1 signaling results in defective cortical development. *Cereb Cortex* 2008; 18: 938-950 [PMID: 17656621 DOI: 10.1093/ercor/bhm312]

110 Choi JW, Herr DR, Noguchi K, Yung YC, Lee CW, Mutoh T, von Kügelgen I, Al Aboud K, Lee YA. Molecular cloning and characterization of a G protein-coupled receptor P2Y5 and its ligand LPA are involved in maintenance of human hair growth. *Pharmacol Res* 2010; 62: 479-488 [PMID: 19796809 DOI: 10.1016/j.phrs.2009.09.035]

111 Sumida H, Noguchi K, Kihara Y, Abe M, Yanagida K, Hamano F, Sato S, Tamaki K, Morishita Y, Kano MR, Iwata C, Miyazono K, Sakimura K, Shimizu T, Ishii S. LPA4 regulates blood and lymphatic vessel formation during mouse embryogenesis. *Blood* 2010; 116: 5060-5070 [PMID: 20713964 DOI: 10.1182/blood-2010-03-272443]

112 Kotarsky K, Boketoff A, Brustul J, Nilsson NE, Norberg A, Hansson S, Owman C, Sillard R, Leeb-Lundberg LM, Olde B. Lysophosphatidic acid binds to and activates GPR92, a G protein-coupled receptor highly expressed in gastrointestinal lymphocytes. *J Pharmacol Exp Ther* 2006; 318: 619-628 [PMID: 16651410 DOI: 10.1124/jpet.105.098848]

113 Lin S, Yeruva S, He P, Singh AK, Zhang H, Chen M, Lampecht G, de Jonge HR, Tse M, Donovitz M, Hogema BM, Chun J, Seidler U, Yun CC. Lysophosphatidic acid stimulates the intestinal brush border Na(+)/H(+) exchanger 3 and fluid absorption via LPA(5) and NHERF2. *Gastroenterology* 2010; 138: 649-658 [PMID: 19800338 DOI: 10.1053/j.gastro.2009.09.055]

114 Lundequist A, Boyce JA. LPA5 is abundantly expressed by human mast cells and important for lysophosphatidic acid induced MIP-1α release. *PLoS One* 2011; 6: e18192 [PMID: 21464938 DOI: 10.1371/journal.pone.0018192]

115 Araki M, Kiyayoshi M, Dong Y, Hirane M, Ozaki S, Mori S, Fukushima N, Honoki K, Tsujiuchi T. Inhibitory effects of lysophosphatidic acid receptor-5 on cellular functions of sarcoma cells. *Growth Factors* 2014; 32: 117-122 [PMID: 24798396 DOI: 10.3109/09771914.2014.911294]

116 Kaplan MH, Smith DI, Sundick RS. Identification of a G protein-coupled receptor induced inactivated T cells. *J Immunol* 1993; 151: 628-636 [PMID: 8393036]

117 Webb TE, Kaplan MG, Barnard EA. Identification of 6H1 as a P2Y purinoceptor: P2Y5. *Biochem Biophys Res Commun* 1996; 219: 105-110 [PMID: 8619790]

118 Lee M, Choi S, Haldén G, Yl J, Schichnes D, Aponte GW. P2Y5 is a G(alpha) protein-coupled receptor activated by lysophosphatidic acid that reduces intestinal cell adhesion. *Am J Physiol Gastrointest Liver Physiol* 2009; 297: G641-G654 [PMID: 19679818 DOI: 10.1152/ajpgi.00199.2009]

119 Pasterнак SM, von Kügelgen I, Al Aboud K, Lee YA, Rüschendorf F, Voss K, Hillmer AM, Molderings GJ, Franz T, Hennes A, Alpers HM, Ovaas H, Moolenaar WH, Lee SJ. Autotaxin/Lpa3 signaling regulates Kupffer’s vesicle formation and left-right asymmetry in zebrafish. *Development* 2012; 139: 4439-4448 [PMID: 23095890 DOI: 10.1242/dev.081745]

120 Rhee HJ, Nam JS, Sun Y, Kim MJ, Choi HK, Han DH, Kim NH, Huh SO. Lysophosphatidic acid stimulates CAMP accumulation and cAMP response element-binding protein phosphorylation in immortalized hippocampal progenitor cells. *Neuroreport* 2006; 17: 525-526 [PMID: 16543818 DOI: 10.1097/01.wnr.0000202011.16718.68]

121 Lee Z, Cheng CT, Zhang H, Subler MA, Wu J, Mukherjee A, Windle JJ, Chen CK, Fang X. Role of LPA4/p2Y9/GPR23 in negative regulation of cell motility. *Mol Biol Cell* 2008; 19: 5435-5445 [PMID: 18843448 DOI: 10.1091/mbc.E07-03-0316]

122 Liu YB, Kharode Y, Bodine PV, Yaworsky PJ, Robinson JA, Billiard J. LPA induces osteoblast differentiation through interplay of two receptors: LPA1 and LPA4. *J Cell Biochem* 2010; 109: 794-800 [PMID: 20069565 DOI: 10.1002/jcb.22471]

123 Mansell JP, Barbour M, Moore C, Nowghani M, Pabbruwe M, Sjostrom T, Blom AW. The synergistic effects of lysophosphatidic acid receptor agonists and calcitriol on MG63 osteoblast maturation at titanium and hydroxyapatite surfaces. *Biomaterials* 2010; 31: 199-206 [PMID: 19796809 DOI: 10.1016/j.biomaterials.2009.03.035]

124 Rhee HJ, Nam JS, Sun Y, Kim MJ, Choi HK, Han DH, Kim NH, Huh SO. Lysophosphatidic acid activates CAMKIV and cAMP response element-binding protein phosphorylation in immortalized hippocampal progenitor cells. *Neuroreport* 2006; 17: 525-526 [PMID: 16543818 DOI: 10.1097/01.wnr.0000202011.16718.68]

125 Lee Z, Cheng CT, Zhang H, Subler MA, Wu J, Mukherjee A, Windle JJ, Chen CK, Fang X. Role of LPA4/p2Y9/GPR23 in negative regulation of cell motility. *Mol Biol Cell* 2008; 19: 5435-5445 [PMID: 18843448 DOI: 10.1091/mbc.E07-03-0316]

126 Liu YB, Kharode Y, Bodine PV, Yaworsky PJ, Robinson JA, Billiard J. LPA induces osteoblast differentiation through interplay of two receptors: LPA1 and LPA4. *J Cell Biochem* 2010; 109: 794-800 [PMID: 20069565 DOI: 10.1002/jcb.22471]

127 Mansell JP, Barbour M, Moore C, Nowghani M, Pabbruwe M, Sjostrom T, Blom AW. The synergistic effects of lysophosphatidic acid receptor agonists and calcitriol on MG63 osteoblast maturation at titanium and hydroxyapatite surfaces. *Biomaterials* 2010; 31: 199-206 [PMID: 19796809 DOI: 10.1016/j.biomaterials.2009.03.035]

128 Rhee HJ, Nam JS, Sun Y, Kim MJ, Choi HK, Han DH, Kim NH, Huh SO. Lysophosphatidic acid activates CAMKIV and cAMP response element-binding protein phosphorylation in immortalized hippocampal progenitor cells. *Neuroreport* 2006; 17: 525-526 [PMID: 16543818 DOI: 10.1097/01.wnr.0000202011.16718.68]
Shinomura Y, Garzon MC, Kristal L, Shapiro L, Christiano AM. Autosomal recessive wooly hair with hypotrichosis caused by a novel homozygous mutation in the P2RY5 gene. *Exp Dermatol* 2009; 18: 218-221 [PMID: 18803659 DOI: 10.1111/ j.1600-0622.2008.00788.x]

Nahum S, Morice-Picard F, Taieb A, Sprecher E. A novel mutation in LPAR6 causes autosomal recessive hypotrichosis of the scalp. *Clin Exp Dermatol* 2011; 36: 188-194 [PMID: 21070332 DOI: 10.1111/j.1365-2230.2010.03944.x]

Archbold JK, Martin JL, Sweet MJ. Towards selective lysophospholipid GPCR modulators. *Trends Pharmacol Sci* 2014; 35: 219-226 [PMID: 24746475 DOI: 10.1016/j.tips.2014.03.004]

Choi JW, Lee CW, Chun J. Biological roles of lysophospholipid receptors revealed by genetic null mice: an update. *Biochim Biophys Acta* 2008; 1781: 531-539 [PMID: 18407842 DOI: 10.1016/j.bbalip.2008.03.004]

Valentine WJ, Fells JI, Perygin DH, Mujahid S, Yokoyama K, Fujiwara Y, Tsukahara R, Van Brocklyn JR, Parrill AL, Tigliy G. Subtype-specific residues involved in ligand activation of the endothelial differentiation gene family lysophosphatidic acid receptors. *J Biol Chem* 2006; 281: 12175-12187 [PMID: 18316373 DOI: 10.1074/jbc.M708847200]

Lee SC, Fujiwara Y, Liu J, Yue J, Shimizu Y, Norman DD, Wang Y, Tsukahara R, Szabo E, Patil R, Barnerjee S, Miller MD, Balazs L, Ghosh MC, Waters CM, Oravez T, Tigliy GJ. Autotaxin and LPA and LPA5 receptors exert disparate functions in tumor cells versus the host tissue microenvironment in melanoma invasion and metastasis. *Mol Cancer Res* 2013; 13: 174-185 [PMID: 25158955 DOI: 10.1158/1541-7766.MCR-12-0602]

Amistren S, Braun OO, Bengtsson A, Erlinge D. Gene expression profiling for the identification of G-protein coupled receptors in human platelets. *Thromb Res* 2008; 122: 47-57 [PMID: 17920662]

Yanagida K, Kurikawa Y, Shimizu T, Ishii S. Current progress in non-Edg family LPA receptor research. *Biochim Biophys Acta* 2013; 1831: 33-41 [PMID: 22902318 DOI: 10.1016/ j.bbalip.2012.08.003]

Yanagida K, Ishii S. Non-Endg family LPA receptors: the cutting edge of LPA research. *J Biochem* 2011; 150: 223-232 [PMID: 21746769 DOI: 10.1093/jb/mvr087]

Dong Y, Hirane M, Araki M, Fukushima N, Tsujutchi T. Lysophosphatidic acid receptor-5 negatively regulates cellular responses in mouse fibroblast 3T3 cells. *Biochim Biophys Acta Gen Comp* 2014; 446: 585-589 [PMID: 24623199 DOI: 10.1016/ j.bjbc.2014.03.016]

UniProt Consortium. Update on activities at the Universal Protein Resource (UniProt) in 2013. *Nucleic Acids Res* 2013; 41: D43-D47 [PMID: 23161681 DOI: 10.1093/nar/gks1068]

Ogden CL, Carroll MD, Fryar CD, Flegal KM. Prevalence of Obesity Among Adults and Youth: United States, 2011-2014. *NCHS Data Brief* 2015; 1-8 [PMID: 26630346]

Reilly JJ, Kelly J. Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review. *Int J Obes (Lond)* 2011; 35: 891-898 [PMID: 20975725 DOI: 10.1038/ijo.2010.222]

Isakson P, Hammarstedt A, Gustafson B, Smith U. Impaired proadipocyte differentiation in human embryonic obesity: role of Wnt, tumor necrosis factor-alpha, and inflammation. *Diabetes* 2009; 58: 1550-1557 [PMID: 19351711 DOI: 10.2337/db08-1770]

Valet P, Pagès C, Jeanneton O, Daviaud D, Barbe P, Record M, Sauder J, Valet P. Lipid lysophospholipid GPCR modulators. *Trends Pharmacol Sci* 2012; 36: 461-470 [PMID: 22287230 DOI: 10.1016/j.tips.2011.05.029]

Yang F, et al. LPA in the regulation of adipocyte functions and liver fibrosis. *J Biol Chem* 2012; 287: 569-577 [PMID: 22466426 DOI: 10.1074/jbc.M112577200]

Benesch MG, Zhao YY, Curtis JM, McMullen TP, Brandley DN. Regulation of autotaxin expression and secretion by lysophosphatidate and sphingosine 1-phosphate. *J Lipid Res* 2015; 56: 1134-1144 [PMID: 25896349 DOI: 10.1194/jlr.M057661]

Lundgren M, Svensson M, Lindmark S, Renström F, Ruge T, Eriksson JW. Fat cell enlargement is an independent marker of insulin resistance and ‘hyperleptinemia’. *Diabetologia* 2007; 50: 625-633 [PMID: 17216279]

Arner E, Westmark PO, Spalding KL, Britton T, Rydén M, Frisen J, Bernard S, Arner P. Adipocyte turnover: relevance to human adipose tissue morphology. *Diabetes* 2010; 59: 105-109 [PMID: 19846802 DOI: 10.2337/db09-0942]

Boucher J, Quilliot D, Pradères JP, Simon MF, Grès S, Guigné C, Privé D, Fery G, Boutin JA, Carpenè C, Valet P, Saudier-Blanche JS. Potential involvement of autotaxin insulin resistance in obesity-associated up-regulation of autotaxin lysophospholipase D/autotaxin expression. *Diabetologia* 2005; 48: 569-577 [PMID: 15700135]
The link between Hepatic Vitamin A Metabolism and liver fibrosis. J Nutr Biochem 1996; 7: 2300-2305 [PMID: 8931461]

Yea K, Kim J, Lim S, Park HS, Park KS, Suh PG, Ryu SH. Lyosphosphatidic acid regulates blood glucose by stimulating myotube and adipocyte glucose uptake. J Mol Med (Berl) 2008; 86: 211-220 [PMID: 17920484 DOI: 10.1007/s00109-007-0269-z]

Barber MN, Rissi S, Yang C, Meikle PJ, Staples M, Febrario MA, Bruce CR. Plasma lyosphosphatidylcholine levels are reduced in Type 2 diabetes. PLoS One 2012; 7: e41456 [PMID: 22848850 DOI: 10.1371/journal.pone.0041456]

Rancoule C, Attané C, Grés S, Fournel D, Dusauley R, Bertrand C, Vinel C, Tréguer K, Prentki M, Veil P, Saulnier-Blache JS. Phosphatidic acid impairs glucose homeostasis and inhibits insulin secretion in high-fat diet obese mice. Diabetologia 2013; 56: 1394-1402 [PMID: 23508306 DOI: 10.1007/s00125-013-2801-3]

D’Souza K, Kane DA, Touabia M, Kershaw EE, Pulimukkunni T, Kienesberger PC. Autotaxin Is Regulated by Glucose and Insulin in Adipocytes. Endocriology 2017; 158: 791-803 [PMID: 28324037 DOI: 10.1210/endo-2017-00035]

Reeves VL, Trybula JS, Wills RC, Goodpaster BH, Dubé JJ, Kienesberger PC, Kershaw EE. Serum Autotaxin/ENPP2 correlates with insulin resistance in older humans with obesity. Obesity (Silver Spring) 2015; 23: 2371-2376 [PMID: 26727116 DOI: 10.1002/oby.201232]

Im DS, Fujioka T, Katada T, Kondo Y, Ui M, Okajima F. The link between Hepatic Vitamin A Metabolism and liver fibrosis. J Nutr Biochem 1996; 7: 2300-2305 [PMID: 8931461]

Yea K, Kim J, Lim S, Park HS, Park KS, Suh PG, Ryu SH. Lyosphosphatidic acid regulates blood glucose by stimulating myotube and adipocyte glucose uptake. J Mol Med (Berl) 2008; 86: 211-220 [PMID: 17920484 DOI: 10.1007/s00109-007-0269-z]

Barber MN, Rissi S, Yang C, Meikle PJ, Staples M, Febrario MA, Bruce CR. Plasma lyosphosphatidylcholine levels are reduced in Type 2 diabetes. PLoS One 2012; 7: e41456 [PMID: 22848850 DOI: 10.1371/journal.pone.0041456]

Rancoule C, Attané C, Grés S, Fournel D, Dusauley R, Bertrand C, Vinel C, Tréguer K, Prentki M, Veil P, Saulnier-Blache JS. Phosphatidic acid impairs glucose homeostasis and inhibits insulin secretion in high-fat diet obese mice. Diabetologia 2013; 56: 1394-1402 [PMID: 23508306 DOI: 10.1007/s00125-013-2801-3]

D’Souza K, Kane DA, Touabia M, Kershaw EE, Pulimukkunni T, Kienesberger PC. Autotaxin Is Regulated by Glucose and Insulin in Adipocytes. Endocriology 2017; 158: 791-803 [PMID: 28324037 DOI: 10.1210/endo-2017-00035]

Reeves VL, Trybula JS, Wills RC, Goodpaster BH, Dubé JJ, Kienesberger PC, Kershaw EE. Serum Autotaxin/ENPP2 correlates with insulin resistance in older humans with obesity. Obesity (Silver Spring) 2015; 23: 2371-2376 [PMID: 26727116 DOI: 10.1002/oby.201232]

Im DS, Fujioka T, Katada T, Kondo Y, Ui M, Okajima F. The link between Hepatic Vitamin A Metabolism and liver fibrosis. J Nutr Biochem 1996; 7: 2300-2305 [PMID: 8931461]
Ikeda H, Yatomi Y, Yanase M, Satoh H, Nishihara A, Kawabata M, Fujiwara K. Effects of lysophosphatidic acid on proliferation of stellate cells and hepatocytes in culture. Biochem Biophys Res Commun 1998; 248: 436-440 [PMID: 9675156]

Yanase M, Ikeda H, Matsui A, Maekawa H, Noiri E, Tomiya T, Arai M, Yano T, Shibata M, Ikebe M, Fujiwara K, Rojkind M, Ogata I. Lysophosphatidic acid enhances collagen gel contraction by hepatic stellate cells: association with rho-kinase. Biochem Biophys Res Commun 2000; 277: 72-78 [PMID: 11027642 DOI: 10.1006/bbrc.2000.3634]

Yanase M, Ikeda H, Ogata I, Matsui A, Noiri E, Tomiya T, Arai M, Inoue Y, Tejima K, Nagashima K, Nishikawa T, Shibata M, Ikebe M, Rojkind M, Fujiwara K. Functional diversity between Rho-kinase- and MLCK-mediated cytoskeletal actions in a myofibroblast-like hepatic stellate cell line. Biochem Biophys Res Commun 2003; 305: 223-228 [PMID: 12745062]

Ikeda H, Nagashima K, Yanase M, Tomiya T, Arai M, Inoue Y, Tejima K, Nishikawa T, Omata M, Kimura S, Fujiwara K. Involvement of Rho/Rho kinase pathway in regulation of apoptosis in rat hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol 2003; 285: G880-G886 [PMID: 12829436]

Gobeil F Jr, Zhu T, Brault S, Geha A, Vazquez-Tello A, Fortier A, Barbaz D, Checchin D, Hou X, Nader M, Bkaily G, Gratton JP, Heveker N, Ribeiro-da-Silva A, Peri K, Bard H, Chorvatova A, D’Orléans-Juste P, Goetzl EJ, Chemtob S. Nitric oxide signaling via nuclearized endothelial nitric-oxide synthase modulates expression of the immediate early genes iNOS and mPGES-1. J Biol Chem 2006; 281: 16058-16067 [PMID: 16574649 DOI: 10.1074/jbc.M602219200]

Tigyi G, Parrill AL. Molecular mechanisms of lysosphosphatidic acid action. Prog Lipid Res 2003; 42: 498-526 [PMID: 14559069]

Yang F et al. LPA in the regulation of adipocyte functions and liver fibrosis
