

REVIEWS

LYSOPHOSPHATIDYLCHOLINE AND LYSOPHOSPHATIDYLINOSIOL
– NOVEL PROMISING SIGNALING MOLECULES AND THEIR POSSIBLE
THERAPEUTIC ACTIVITY

ANNA DRZAZGA, AGATA SOWIŃSKA and MARIA KOZIOLKIEWICZ*

Lodz University of Technology, Institute of Technical Biochemistry,
Stefanowskiego 4/10, 90-924 Łódź, Poland

Abstract: For many years the role of lysophospholipids (LPLs) was associated only with structural and storage components of the cell without any informational function. Today, based on many research projects performed during the last decades, it is clear that some of the LPLs act as hormone-like signaling molecules and thus are very important inter- and intracellular lipid mediators. They can activate specific membrane receptors and/or nuclear receptors regulating many crucial physiological and pathophysiological processes. The LPLs were identified as involved in a majority of cellular processes, including modulation of disease-related mechanisms observed, for instance, in case of diabetes, obesity, atherosclerosis and cancer. Among LPLs, lysophosphatidylcholine (LPC) and lysophosphatidylinositol (LPI) are becoming attractive research topics. Their recently revealed activities as novel ligands of orphan G protein-coupled receptors (i.e., GPR55 and GPR119) involved in modulation of tumor physiology and insulin secretion seem to be one of the most interesting aspects of these compounds. Moreover, the most recent scientific reports emphasize the significance of the acyl chain structure bound to the glycerol basis of LPL, as it entails different biological properties and activities of the compounds.

Keywords: lysophospholipids, lysophosphatidylcholine, lysophosphatidylinositol, GPR55, GPR119, diabetes, cancer

Abbreviations: AMD – age-related macular degeneration, ATX – autotaxin, cAMP – 3'-5'-cyclic adenosine monophosphate, CMC – critical micelle concentration, cPA – cyclic phosphatidic acid, DDHD1 – DDHD domain-containing protein 1, ERK – extracellular signal-regulated kinase, GAPDH – glyceraldehyde 3-phosphate dehydrogenase, GIP – glucose-dependent insulinotropic peptide, GLP-1 – glucagon-like peptide 1, GM-CSF – granulocyte macrophage colony-stimulating factor, GPCR – G protein-coupled receptors, GPLs – glycerolysophospholipids, IFN- γ – cytokine-induced interferon γ , IL – interleukin, LCAT – lecithin:cholesterol acyltransferase, LDH – lactate dehydrogenase, LDL – low-density lipoprotein, LPA – lysophosphatidic acid, LPE – lysophosphatidylethanolamine, LPG – lysophosphatidylglycerol, LPS – lysophosphatidylserine, LSPL – lysosphingophospholipid, lysoPLD – lysophospholipase D, NADPH – nicotinamide adenine dinucleotide phosphate, PC – phosphatidylcholine, PGE₂ – prostaglandin E₂, PGF1 α – 6-keto-prostaglandin F_{1 α} ; PGI₂ – prostaglandin I₂ (prostacyclin), PI – phosphatidylinositol, PLA₁ – phospholipase A₁, PLA₂ – phospholipase A₂, PLC – phospholipase C, PPAR γ – proliferator-activated receptor γ , ROS – reactive oxygen species, S-1-Ps – sphingosine-1-phosphates, SPC – sphingosylphosphatidylcholine, T1DM – type 1 diabetes mellitus, T2DM – type 2 diabetes mellitus, TGF- β 1 – transforming growth factor β 1, TRPM8 – transient receptor potential cation channel subfamily M member 8, TRPV2 – transient receptor potential cation channel subfamily V member 2, TXA₂ – thromboxane A₂

Lysophospholipids (LPLs) for a long time were considered only as membrane components necessary to mediate synthesis of various phospholipids and to embed proteins into cell membranes. Thanks to the rapid advance in chemistry and biology of LPLs during the last decades, the group of “bioactive lysophospholipids” of hormone-like sig-

naling properties was revealed. Most popular in this area are LPLs with glycerol (glycerolysophospholipids, GPLs) or sphingoid (lysosphingophospholipids, LSPLs) backbones, where families of lysophosphatidic acids (LPAs) and sphingosine-1-phosphates (S-1-Ps) have been investigated to the greatest extent so far. Much less information is

* Corresponding author: e-mail: maria.koziolekiewicz@p.lodz.pl, phone: (0-42) 631 34 03, fax: (0-42) 636-66-18

available considering the remaining LPLs, such as cyclic phosphatidic acid (cPA), lysophosphatidylglycerol (LPG), sphingosylphosphatidylcholine (SPC), lysophosphatidylserine (LPS), lysophosphatidylethanolamine (LPE), lysophosphatidylcholine (LPC) and lysophosphatidylinositol (LPI). Glycerol derivatives of lysophospholipids are a diverse group of molecules bearing both saturated (e.g., 16:0, 18:0) and unsaturated (e.g., 18:1, 18:2, 20:4) fatty acid chains, contrary to SIP (2*S*-amino-1-(dihydrogen phosphate)-4*E*-octadecene-1,3*R*-diol), which is a single molecular species. Common structural features of GPLs is a glycerol backbone, a phosphate head group at the *sn*-3 position, a hydroxyl group at the *sn*-2 (or *sn*-1) position and a single fatty acid chain at the *sn*-1 (or *sn*-2) position. Besides, the acyl chain at the *sn*-2 position of the 2-acyl-lysophospholipid has a tendency to migrate to the *sn*-1 position, thus resulting in the creation of the 1-acyl-lysophospholipid (1, 2).

Lysophospholipids are necessary to maintain homeostasis of many physiological processes including reproduction, vascular development, and functioning of the nervous system. The majority of the studies have demonstrated beneficial effects of particular lysophospholipids towards health. The famous example of a brain permeant LPL, which was officially approved by US Food and Drug Administration and European Medicines Agency as the first orally administered drug in multiple sclerosis treatment, is 2-amino-2-[2-(4-octylphenyl)ethyl]-1,3-propanediol (FTY720, the SIP analogue known as *fingolimod* and, recently, *Gilenya*) (3). Another example is *edelfosine* (1-octadecyl-2-*O*-methylglycero-3-phosphocholine, 2-LPC) which is a drug proposed in treatment of multiple sclerosis and other neurodegenerative diseases (4). The pharmacological research results are the best proof of favorable activities of LPLs and their great potency in disease therapy.

Currently, LPC and LPI seem to be the most attractive research goals in terms of biological activity and possible application. In serum plasma both of the compound groups function mainly as substrates for autotaxin (ATX). Enzymatic digestion of LPC and LPI leads to formation of various forms of LPA and cPA, which are involved i.a., in modulation of cardiovascular system physiology, wound healing, metabolism of lipids and carbohydrates, mediated by membrane and nuclear receptors (respectively: receptors of LPA family and nuclear peroxisome proliferator-activated receptor γ , PPAR γ) (5). However, according to recent findings, both LPC and LPI are capable of modulation of sig-

nificant physiological processes directly due to interaction with respective G protein-coupled receptors (GPCRs), which at the same time identify them as novel ligands of "orphan receptors".

LPC (1-acyl-glycero-3-phosphocholine) is known for its favorable effects towards health in terms of indirect promotion of internal and external wound healing and organ regeneration (5, 6), therapy of autoimmune and neurodegenerative diseases (4, 7–9) or even treatment of age-related macular degeneration (AMD) (10). Moreover, recent reports have shown that LPC induces insulin secretion from pancreatic β -cells. It has been also found that LPC activates glucose uptake and effectively lowers blood glucose levels in mouse models of type 1 and 2 diabetes mellitus (T1DM and T2DM, respectively) (11, 12). Unfortunately, up to date, the precise mechanism of this phenomenon has remained unknown. On the other hand, oleoyllysophosphatidylcholine (LPC 18:1) was reported to bind to one of membrane receptors known as GPR119, which is responsible for increase in glucose stimulated insulin secretion in murine NIT-1 insulinoma cells due to protein kinase A (PKA) related signaling pathway (12, 13). The nature of the endogenous ligands to GPR119, and whether this GPCR plays a physiological role in direct regulation of insulin secretion by pancreatic β cell, is currently under investigation.

When it comes to LPI (1- α -lysophosphatidylinositol), there is a strong evidence that it is involved in tumor cell proliferation and migration. Clinical data identified LPI as a biomarker for poor prognosis in cancer patients, whereas *in vitro* studies demonstrated significantly elevated levels of LPI in highly proliferative cancer cells (14). In 2007, Oka et al. documented for the first time that LPI is an agonist of GPR55 expressed to high extent by tumor cells. The team found LPI to induce rapid phosphorylation of the extracellular signal-regulated kinase (ERK) and an increase in intracellular Ca^{2+} in GPR55-expressing cells. On the other hand, there is a lot of controversies about natural ligands for GPR55, which need to be clarified.

All known LPLs characterized by various biological activities need to be further studied in detail. One of the issues to be clarified is the mechanism of action of the biomolecules with respect to their specific chemical structure. This review is especially devoted to activities and mechanisms of action of LPC and LPI species recently discovered as meaningful signaling molecules. The subject is being intensively studied by the research team of the Institute of Technical Biochemistry, Lodz

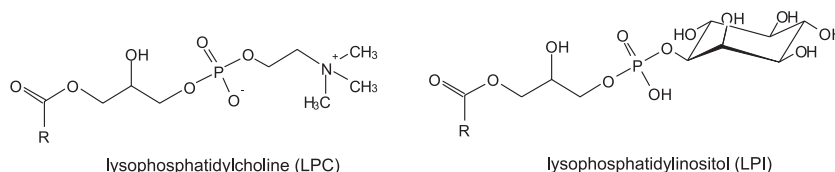


Figure 1. Structure of lysophosphatidylcholine (LPC) and lysophosphatidylinositol (LPI). „R“ represents a fatty acid residue

University of Technology (IBT LUT) in Poland (2, 15).

Lysophosphatidylcholine

LPC is the most abundant LPL with relatively high concentration in human blood (approximately 150 mM), however, showing significant level variations with respect to gender and age (16). To some extent, it is available in dietary products (i.e., eggs, soya, oilseeds and fish). Most of the circulating LPC molecules are associated with albumin. They are also the major phospholipid component of oxidized low-density lipoproteins (17, 18). Several types of LPC molecules with various acyl chains, such as palmitoyl (16:0), stearoyl (18:0), oleoyl (18:1), linoleoyl (18:2), arachidonoyl (20:4) and docosahexanoyl (22:6), have been found in human plasma (19). The compounds are derived from phosphatidylcholine due to transacylation of the sn-2 fatty acid residue of lecithin to free cholesterol catalyzed by lecithin:cholesterol acyltransferase (LCAT), which finally results in the formation of cholesterol ester and LPC (1, 20). The rate of ester formation by LCAT depends on the nature of the head group, fatty acid residues and the macromolecular properties of the lipid (1). It is also generated by the action of phospholipases A₂ (PLA₂) and phospholipases A₁ (PLA₁), which are able to cleave the sn-2 and sn-1 ester bond, respectively (21). Appreciable amounts of LPC are also formed in plasma by endothelial lipase (1, 17).

Structurally different LPCs were recognized as carriers of fatty acids, phosphatidylglycerol and choline between tissues (22). As a representative of pro-inflammatory LPLs, LPC is involved in modulation of T cell functions and immunity. In activated microglia (brain macrophages) LPC was found to trigger interleukin-1 β (IL-1β) processing and release (23). It was also reported to enhance the expression of cytokine-induced interferon γ (IFN-γ) (1) and transforming growth factor β1 (TGF-β1) (24). Moreover, LPC-dependent NADPH oxidase

stimulation and production of reactive oxygen species (ROS) was documented to activate caspase-1 that converts pro-cytokines to their mature, biologically active forms (IL-1β, IL-18 and IL-33) (25). LPC is also involved in the production of prostaglandin I₂ (PGI₂ also known as prostacyclin) *in vitro* in primary human aortic endothelial cells and *in vivo* in murine model. A stable degradation product of PGI₂, 6-keto-prostaglandin F_{1α} (PGF_{1α}) was also found induced by LPC species (namely 18:1 and 20:4) (1).

In addition, it was recently found out that polyunsaturated LPC variants, such as LPC 20:4 and LPC 22:6, may serve as successful anti-inflammatory agents, opposing the activity of saturated acyl LPCs known to induce the response of immunological system (i.e., LPC 16:0) (8, 26, 27). As presented by Jin et al., LPC 22:6 as well as 17-hydroxy-LPC 22:6 exert anti-inflammatory effect in mice treated with lipopolysaccharide. It was detected that administration of these compounds before injection of lipopolysaccharide reduces increase in weight of spleen dose-dependently, where 17-hydroxy-LPC 22:6 appears to be more effective (9). Another example of anti-inflammatory polyunsaturated LPC is 15-hydroxy-docosapentanoyl-LPC (15-hydroxy-LPC 20:5) that inhibits formation of leukotrienes and cytokines in zymosan A-induced peritonitis of mice (28). Similar observation was made in case of LPC 20:4 and its derivative (15-hydroxy-LPC 20:4) (29). The anti-inflammatory effects were related to down-regulation of leukocyte extravasation, plasma leakage, and formation of pro-inflammatory mediators (IL-5, IL-6, nitric oxide, 12-hydroxyeicosatetraenoic acid and PGE₂ stimulated by LPC 16:0), and up-regulation of anti-inflammatory mediators (IL-4 and IL-10) (27). This evidence suggests that LPCs could be regarded as major modulators of inflammation process. LPC, together with LPS and LPE, is also found to be a natural agonist of G2A receptor, which in this case serves as suppressor of autoimmunity (1, 30). LPC, LPS and LPE, regard-

less of differences in structure, induce mobilization of intracellular Ca^{2+} ions through G2A signaling-dependent pathway. However, it resulted from amphipatic nature and detergent-like properties of the compounds and their interaction with lipid bilayer of the cellular membrane rather than direct interaction of the LPLs with the receptor. As originally described by Ben-Zeev et al., the process leads to $G_{\alpha i}$ -related activation of phospholipase C (1, 31). On the other hand, direct, receptor mediated release of $G_{\alpha q/11}$ subunit without the membrane permeabilization effect was also observed (1, 32).

The problem of membrane permeabilization can be a matter of cytotoxicity. LPC above critical micelle concentration (CMC) can lead to rapture of cellular membrane resulting in hemolysis (1). However, there can be over 200-fold difference in CMC/toxic value depending on the structure of the LPC's acyl residue (33, 34). The issue was studied for the first time for hexanoyl (6:0), octanoyl (8:0), decanoyl (10:0), 12:0, 14:0, 16:0, 18:0, 18:1, nonadecanoyl (19:0), arachidoyl 20:0, and lignoceroyl 24:0 LPC species by Kim et al. in 2007 (Jurkat cell model) (35). The research led to identification LPC with palmitoyl residue as the most toxic. Yet, applied concentration of all tested LPCs was 20 μM , which is much higher than estimated CMC values of these compounds (33, 34). It was also found that serum albumin is capable of reduction of any cytotoxic effects caused by LPLs (35).

Six years later, Rytczak et al. at IBT LUT performed synthesis of novel phosphorothioate and phosphorodithioate analogues of 2-methoxylysophosphatidylcholine 12:0, 16:0, 18:0 and 18:1, which was followed by assessment of their influence on viability and cell membrane integrity of β -TC3 murine insulinoma cell model (2). The biological studies addressed possibility of engagement of LPC species in modulation of insulin secretion. Diversity of investigated LPC structures allowed the team to indicate the dependence of strength of the observed biological effects on chemical modification and acyl chain bound to the glycerol backbone (sample results in Table 1). The cytotoxicity assessment involved only a 10- μM concentration, which for some of the tested species was very distant from the expected CMC (i.e., $\text{CMC}_{\text{LPC } 12:0} = 740 \mu\text{M}$, $\text{CMC}_{\text{LPC } 18:0} = 0.4 \mu\text{M}$) (2, 33, 34). Surprisingly, it was clearly depicted that none of the tested compounds caused any significant decrease in cell viability, where the strongest toxic effect (relative cell viability > 72%) was observed for native LPC 14:0 and LPC 16:0, which is in contrast to results obtained by Kim et al. (2). It is worth noticing that the experi-

ments were based on serum-free culture media. What is more, some of the compounds were found promoting cell survival (i.e., LPC 12:0, LPC 18:1), which was also observed in case of some methoxy-LPC's modified with one or two sulfur atoms, which native counterparts are toxic (LPC 14:0 and LPC 16:0). Additionally, the possibility of membrane perturbation by LPC molecules was controlled by assessment of lactate dehydrogenase (LDH) release, which led to a conclusion that the tested LPC analogues at 10 μM do not rapture lipid bilayer regardless of their structure and chemical modification and/or expected toxic CMC (Table 1) (2).

Research group of Rao et al. was also interested in activity of different LPCs with respect to their specific structure. The group investigated LPC 16:0, 18:1, 18:2 and 20:4 in terms of their influence on vasorelaxation. Table 1 presents sample data on prostanoid release from LPC-treated (10 μM concentration) aortic rings. Detection included $\text{PGF}_{1\alpha}$, PGE_2 , PGI_2 and thromboxane A2 (TXA2) (36). As can be noticed from Table 1, different forms of LPC caused significantly different effects on the same experimental model. For instance, LPC 20:4 was capable of prostanoid release induction at 5-fold greater level than LPC 16:0. Taking into account all the stated research on varied LPC activities, it can be surely stated that there are plenty of distinct biological effects exerted by LPCs depending on their specific structure. (2, 35, 36).

Despite obvious connection between acyl chain structure and biological impact of LPC species, the concept of research regarding this issue is neglected in many cases. As far as the role of LPC in diabetes is concerned, the only documented activity concerns LPC 18:1 (13). This LPC species was defined as a novel ligand of GPR119 – a key receptor responsible for regulation of insulin secretion from β cells of pancreatic islets (13, 37), which is also engaged in reduction of fat deposition and food intake. GPR119 is expressed in the human Langerhans islets at the level of 8% relatively to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (38). Its activation entails intracellular cAMP accumulation connected to release of $G_{\alpha s}$ protein (12, 13). Expression of this receptor was also documented in case of enteroendocrine cells of the gut (12, 39–41), where its activation was related to stimulation of glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). This clearly shows that GPR119 is involved both directly and indirectly in the process of insulin secretion. What is more, since GLP-1 promotes expansion of β -cell mass, it is possible that agonists

Table 1. Comparison of biological impact of structurally different LPCs. Assessment of cytotoxicity, membrane perturbation potential (2) and prostanoid release stimulation (36). ? - no available data.

LPC modification	<i>In vitro</i> cell viability assessment (βTC3 model, 24-hour incubation with 10 μM of LPC, relative %)				LDH release		CMC of native LPC (μM) (33, 34)	Prostanoid release from aortic rings (1-hour incubation with 10 μM of LPC, approximate relative fold of release)			
	native	2-metoxyposphoro		native	-thioate	2-metoxyposphoro- -dithioate		6-keto-PGF _{1α}	TXB ₂	PGF _{2α}	PGE ₂
		-thioate	-dithioate								
LPC 12:0	~106	~90	~87	<0	<0	<5	740				?
LPC 14:0	~75	~112	~104	<0	<0	<5	45				?
LPC 16:0	~73	~105	~113	<0	<5	<5	7	4	1.67	1	1.5
LPC 18:0	~89	~90	~86	<0	<0	<0	0,4				?
LPC 18:1	~130	~121	~91	<0	<0	<0	10	4.5	2.67	1.67	3
LPC 18:2				?				5	2	1	3
LPC 20:4				?				21	5	4.83	7

of GPR119 may influence both the secretory activity and the viability of β-cells at the same time (40, 41). Properly chosen ligand of GPR119 may thus lead to improved glucose homeostasis in patients with T2DM, which is currently affecting over 90% of the patients due to severe insulin resistance and defective insulin secretion (42, 43).

Apart from obviously favorable effects towards T2DM patients, LPC was also found to improve glucose uptake in mouse models of T1DM, which is an insulin independent form of the disease due to autoimmune destruction of pancreatic β cells (11, 42, 43). This means that induction of insulin secretion from β cells, which are destroyed in case of T1DM, is not the only activity of LPC involved in modulation of metabolism.

LPC is the most abundant lysophospholipid in human blood. Significant decrease of its serum concentration was found to be related with high fat diet and T2DM condition (44, 45). These observations are consistent with the previous findings of Soga et al., whose publication clearly shows that LPC is involved in modulation of cellular glucose uptake (13). However, there are also opposite findings where high-fat diet related obesity was correlated with increase of palmitoleyl LPC (LPC 16:1) and LPC 22:4 serum concentration (46) and LPC 18:0 could be even regarded as a biomarker positively related to pathological gain in weight (47).

In the context of regulation of pancreatic hormonal secretion it is worth to pay attention to α cells of Langerhans islets, which are also reported to express GPR119 receptor (48). As far as α cells are responsible for glucagon secretion, activity of which is antagonistic to insulin, it suggests crucial role of GPR119 in maintenance of carbohydrate metabolism, as well as possibly bipolar biological activity of its ligands (38). So far, the potential role of LPC species in glucagon secretion modulation remains unknown.

When regulation of insulin secretion is concerned, GPR55 is another current research topic. Romero-Zerbo in 2011 (49) proposed GPR55 as a novel target for

treatment of T2DM as it was found directly involved in regulation of insulin secretion from β cells. This receptor is found commonly expressed in various tissues of the body, including the islets of Langerhans, however, it is not distributed on α cells of pancreas (50). Oleoylethanolamide (OEA), which is common also for GPR119, is one of the ligands of GPR55 causing enhanced secretion of insulin in *in vitro* cell models (51). There are very little literature data on possible interaction of GPR55 with LPC (1).

Both diabetes types remain incurable to a high extent resulting in indispensable life-long treatment and commonly occurring disease complications (such as hyperglycemia, obesity, cardiovascular and gastrointestinal disorders). This devastating disease affects over 371 million people in the world and is predicted to be the 7th leading cause of death in 2030 (WHO data). When it comes to Poland, it is the fourth European country with the highest epidemiological rates with 3 million people suffering from diabetes, where over one-third is not even aware of the fact. The obvious need of novel diabetes treatment and prophylaxis methods cause the GPR119-LPC interaction an important research area and are expected to expand in the future. It is also a significant matter of studies performed by research teams of IBT LUT.

Beneficial effects of LPC involve regulation of intestinal uptake of nutritional substances. Research results from *in vitro* (e.g., human intestinal Caco-2 cell model) and *in vivo* studies (rat plasma analysis) indicate that mixed micelles containing LPLs significantly influence intestinal uptake of β -carotene and lutein (10, 52). Particularly, micelles containing PC and LPC predominant within the composition cause meaningful increase in uptake of these substances. This is especially important from the point of view of treatment of diseases resulting from nutritional deficiencies, such as age-related macular degeneration (AMD). In this case, an inadequate intake of animal- and plant-based foods with low-fat content was considered to be the major cause of this disease. It was clinically tested that an increased intake of lutein is positively correlated to increased macular lutein density and inversely correlated to the progress of AMD pathogenesis (10). Rodriguez-Navarro et al. emphasize that chronic exposure to high-fat, LPL-rich diet can significantly influence the process of aging and related pathogenesis as dietary lipids are found to modulate the process of chaperone-mediated autophagy, involved in intracellular quality control and response to stress conditions (53).

Dietary available LPC was also found favorable to human health as it was recently published

that LPC species administered to diabetic patients of high risk of cardiovascular disease development successfully decreases serum concentration of LPA responsible for platelet aggregation and vein clotting (54).

Moreover, impaired synthesis of LPC and PC in case of Alzheimer's patients suggests beneficial effects of these lipids in the disease therapy (7). It was also revealed that schizophrenic patients are characterized by significantly diminished levels of LPCs in blood plasma, which may influence the psychosis development as well as result in enhanced susceptibility to infections (55).

From the point of view of animal husbandry, activity of dietary-uptaken LPC is particularly favorable. It is known, that supplementation of fat-based forage for broiler chickens with LPC as an emulsifier improves the gain of their body weight during the starter period (56, 57). Next to increase of total tract apparent digestibility of fatty acids, LPC is also found to cause no adverse effects on the animals' physiology. Focusing on molecular mechanisms associated with the process, LPC is found to induce secretion of enzymes responsible for absorption and transport of dietary lipids. Nakano and co-workers present that oil-feeding accompanied by mixed lipid micelles build of LPC molecules result in 10-fold increase in production of intestinal alkaline phosphatase by the brush border microvilli as compared to the enzyme release in regular high-fat diet conditions (experiments *in vitro*, Caco2 cell line) (58).

LPC was also found to be connected to pathological conditions related to atherosclerosis. It is thought to play a significant role in the atherogenic disease, being a component of oxidized low-density lipoprotein (LDL) in atherosclerotic lesions. LPC 16:0 also induces human umbilical vein endothelial cells (HUVECs) and vascular smooth muscle cells (VSMCs) cell apoptosis processes, which can be associated with atherogenesis (59). On the other hand, there are some evidence suggesting that LPC could be directly engaged in tissue regeneration, as it interacts with LPC GPR4 receptor, which is widely expressed in various endothelial cells and involved in stimulation angiogenesis and cellular migration (30).

Among other properties, LPC is being a natural airway surfactant (60, 61), enabling to enhance viral gene transfer in animal models. Cmielewski and co-workers examined the effect of airway pretreatment with variants of LPC on lentiviral vector gene transfer efficiency in murine nasal airways *in vivo*. Only one hour after administration of LPC

variants was enough to induce an airway barrier function. It can be correlated to the effectiveness of gene expression, where the variants with longer acyl chains appeared to be more effective. The enhanced expression correlated with LPC could provide new options for preclinical development of efficient airway gene transfer techniques.

Lysophosphatidylinositol

LPI is a subspecies of LPL containing an inositol head group. It was found present in different ranges in normal human cells (i.e., endothelial cells (62), platelets (63), peripheral blood neutrophils (64)), various cancer cell lines (65–72), and animal cells (i.e., mouse fibroblasts (73) and macrophages (74), rat brain cells (75)). Concentration of LPI is different in various tissues (37.5 nM per gram of tissue in rat brain, 2.5 μ M in mouse serum and 1.5 μ M in samples of plasma from healthy women (1)) but the fatty acid composition of LPI is distinctive in every mammalian cell type. Derivatives of stearic and arachidonic acids are supposed to be the most active and abundant species. For instance, 2-arachidonoyl (2-20:4) LPI has been reported to have the highest level of biological activity (75).

LPI is a product of phosphatidylinositol (PI) degradation, which is catalyzed by phospholipase PLA₂ (1). Moreover, it was reported that PLA₁ can be also involved in formation of LPI. Yamashita et al. identified phospholipase DDHD domain-containing protein 1 (DDHD1, one of members of the mammalian intracellular PLA₁ family) as a candidate enzyme involved in LPI formation. It was demonstrated that purified DDHD1 is capable of generating 2-arachidonoyl LPI (76).

Up to date, little is known about the export system of LPI. According to the report of Yamashita et al., most of LPI produced by DDHD1 is released into the medium but it was impossible to identify the mechanism of this process (76). However, it has been already found that the ATP-binding cassette ABCC1 transporter is engaged in export of LPI into the extracellular media in case of human prostate cancer PC3 cell line (71).

Deacetylation of LPI by lysophospholipase A (lysoPLA) was the first known degradation pathway of this compound species. The process has been observed in porcine platelets membranes (77). It was also found that degradation of LPI may be catalyzed by phospholipase C (PLC) with specificity for LPI (lysoPI-PLC) (77, 78). Another enzyme involved in LPI metabolism is lysophospholipase D (lysoPLD, autotaxin), which is present in blood plasma and serum and converts LPI to LPA (1, 79).

Physiological role of LPI is still poorly understood. Even though the first publications concerning plausible biological activity of LPI were published in the mid-80s (80, 81), little attention was drawn to this bioactive lipid comparing to LPA or S1P. The research group of Oka was the first to demonstrate significant biological activity of LPI, which only in 2007 was described as the most active endogenous agonist of orphan GPR55 – the intrinsic receptor for LPI (82). At present, LPI is suggested to be able to activate other GPCRs as well. For instance, LPI was reported as an activator of human GPR119 in RH7777 rat hepatoma cells stably expressing this receptor (13).

First reports devoted to biological activity of LPI suggested that the compound is involved in stimulation of insulin release from pancreatic islets. Metz et al. found that the process was mediated *via* intracellular Ca²⁺ ions mobilization (80, 81). These results have indicated that LPI may play an important role in regulation of the whole body metabolism, also in case of diseases such as obesity and T2DM. The thesis was verified during further studies showing increased level of LPI in plasma of obese patients. Moreover, GPR55 expression is enhanced in adipose tissue of obese subjects. Correlation between expression of GPR55 and weight, body mass index and percentage fat mass was also found, which was particularly noticeable in case of women (83). LPI was also reported to increase intracellular calcium level and expression of lipogenic genes in visceral adipose tissue explants and in differentiated visceral adipocytes (1).

Other biological activities of LPI are related to artery contraction, cell proliferation and migration (84). LPI impact on intracellular free Ca²⁺ concentration was analyzed by Oka et al. using GPR55-expressing HEK293 cell model. What is more, Monet et al. demonstrated that application of LPI induces sustained cytoplasmic Ca²⁺ concentration increase in CHO cells, which stably express murine transient receptor potential cation channel subfamily V member 2 (TRPV2) (85). LPI was also reported to activate Akt and enhance intracellular Ca²⁺ levels in prostate and ovarian cancer cells (86). Degradation products of LPI were not observed to induce mobilization of Ca²⁺ or phosphorylation of ERK in GPR55-expressing cells. This proves that the effects of LPI are due to itself, not its metabolites (82). Some recent studies have reported possibility of Ca²⁺ influx induction by LPI through ion channels belonging to the transient receptor potential (TRP) family (87, 88). LPI was found to evoke an increase in intracellular Ca²⁺ in CHO cells expressing murine

transient receptor potential cation channel subfamily M member 8 (TRPM8). The receptor is mainly expressed in a subpopulation of dorsal root ganglion (DRG) neurons that also express GPR55 (89). Other studies have shown that LPI induces Ca^{2+} entry in PC3 cells expressing TRPV2. Induction was observed even in the presence of capsaicin, an inhibitor of TRPV1, which proves that LPI directly interacts with endogenous TRPV2 channel of the PC3 model. This observation suggests that LPI influenced Ca^{2+} influx due to translocation of TRPV2 protein to plasma membrane. However, Ca^{2+} increase induced by LPI *via* TRPV2 channel was observed after some delay, which implies that LPLs are involved in indirect activation of TRPV2 channels, unlike in case of lipid ionotropic receptors (85). On the other hand, it was demonstrated that LPI/GPR55-exerted effects of sustained, oscillatory Ca^{2+} release stimulation are dependent on $\text{G}\alpha_{13}$ and require activation of rhoA (90). It was also reported that effect of LPI in mobilization of intracellular Ca^{2+} in PC3 cells entails migration and proliferation of these cells (71, 85).

The role of LPI is not limited to stimulation of Ca^{2+} mobilization pathway. The compound was observed to be secreted at high concentrations by fibroblasts and epithelial cancer cells, in case of which it showed mitogenic activity (91). LPI was proposed to play an important role in bone physiology due to regulation of osteoclast number and function. Whyte et al. confirmed high level of GPR55 expression in murine and human osteoclasts, implying involvement of LPI in stimulation of osteoclast polarization and bone resorption (92). It was also proven that under inflammatory conditions LPI is produced by immune cells (i.e., macrophages) and involved in stimulation of immunological response (91). High levels of GPR55 expression were observed in spleen (93, 94), neutrophils (91) and mast cells (72).

Interestingly, LPI was recognized as a regulator of peripheral sensory neuron function as well as pathological pain (95). As previously mentioned, LPI stimulates the increase in intracellular Ca^{2+} concentration in DRG neurons and activates TRPM8 channel expressed in this group of neurons. Therefore, it is able to affect DRG neurons involved in nociception, associated with neuropathic or inflammatory pain (89). The same conclusions were drawn by studies on GPR55 knock-out mice, which served as models of inflammatory mechanical hyperalgesia achieved by injection of Freund's complete adjuvant and partial nerve ligation. In this case, the knock-out murine model response to LPI stimu-

lations was found attenuated, which was further attributed to increase in levels of IL-4, IL-10, IFN γ and granulocyte macrophage colony-stimulating factor (GM-CSF). GPR55 knock-out mice also failed to develop mechanical hyperalgesia in the model of neuropathic hypersensitivity (96). What is more, LPI is supposed to possess neuroprotective activity, as it can activate the 2-pore domain K^{+} channels TREK-1 and TRAAK, which are important in terms of synaptic function modulation due to regulation of the neuron membrane potential. This activity does not seem to involve GPCR associated signaling, suggesting a non-receptor mediated activity of LPI. Similar effects were also observed in case of LPC (86).

Detection of GPR55 in various human cancer cell lines, including the ones obtained from breast (68, 70), brain, cervix, skin, pancreas, liver (70), ovaries, prostate (71) and hematological tumors (69, 70, 72), caused increased interest in the role of LPI/GPR55 in cancer progression. Clinical data implicated engagement of LPI in cancer progression and recurrence (84), which was additionally supported by research results showing increased LPI levels in plasma and ascites from ovarian cancer patients as compared to samples from healthy controls or patients with non-malignant diseases (97). Correlation between GPR55 expression in breast cancer tissue and tumor aggressiveness is also known (70). Moreover, similar observation was made during analysis of microarray data concerning pancreatic tumors and glioblastomas (76).

It has been already proven that LPI induces cell migration present in case of physiological and pathophysiological processes, such as embryonic development, immune system activities, inflammatory processes, wound healing, angiogenesis, cancer progression and metastasis (98, 99). Among other activities LPI enhances motility of sperm cells (100), induces directional migration of human peripheral blood neutrophils with improvement to their migratory capacity towards 2-AG (the CB $_2$ R agonist) (91) and exerts pro-migratory influence on human coronary artery smooth muscle cells (SMC) (101). The compound is also found to induce an increase in migration of PC3 cells after activation of TRPV2 channel (85). Migration of highly metastatic MDA-MB-231 human breast adenocarcinoma cells was found significantly enhanced due to LPI treatment as well (68). Interestingly, Ford et al. observed that GPR55 expression in MDA-MB-231 cells is relatively high as compared with poorly metastatic MCF-7 human breast adenocarcinoma cells, however, GPR55 overexpression in MCF-7

cells results in acquirement of their migration ability. Further addition of exogenous LPI enhanced the observed effect even more. The results described here suggest that GPR55 overexpression induced a pro-migratory phenotype in breast cancer cells and that LPI may mediate pro-migratory effects *via* GPR55 activation. Beside the evidence that LPI enhanced breast cancer cell chemotaxis, it was also reported that it can increase cellular polarization and orientation of nanogrooved substratum (68). Nevertheless, there are some contradictory findings on LPI considered as a negative regulator of migration, which was demonstrated in case of endothelial cells (EC) (102). LPI was also shown to stimulate expression of adhesion molecules by endothelial cells (i.e., VCAM-1 in rabbit aortic EC and ICAM-1 in human umbilical vein cells) (103).

The first report indicating the role of LPI in cell proliferation was based on studies devoted to transformation of rodent fibroblasts with cytoplasmic and membrane-associated oncogenes. The process was followed by an increase in intracellular levels of glycerophosphoinositol (GPI), which is a product of LPI deacetylation catalyzed by PLA2 (104). However, the first evidence of direct impact of LPI on cell proliferation was demonstrated by Piñeiro et al., who described mitogenic activity of LPI inducing proliferation of FRTL5 differentiated epithelial thyroid cells. Moreover, comparison of LPI levels in differentiated FRTL5 cells and Kiki cells stably transformed with the k-ras oncogene showed increased concentration of the compound in the latter model (71). Similar results were obtained by Ross et al., who observed highly elevated levels of LPI species and LPI metabolites in cells transformed to overexpress ras-p21 (84). Importantly, enhanced level of LPI was proven to be a consequence of activation of enzymes involved in the Ras pathway, not a transformation itself (105). LPI was also detected in the extracellular medium of ras-transformed fibroblasts, which proves the cells to be capable of both synthesis and release of the lipid (106). Increased levels of intracellular and extracellular LPI were observed in k-ras transformed KiKi cells as well. These results indicate that cancer cells are able to promote their own proliferation by synthesis and release of LPI. The observation is additionally supported by the data on the mechanism of LPI export in PC3 cells, which was found to be mediated by the ABCC1 transporter (71).

Altogether, LPI is a mitogen stimulating proliferation of normal and oncogene-transformed cells. Furthermore, LPI/GPR55 interaction is found pivotal in maintenance of autocrine loop that sus-

tains prostate cancer cell proliferation (71). The observation is consistent with data on overexpression of GPR55 regarded as a strategy of cancer cells to increase their proliferation, which was demonstrated both *in vitro* and *in vivo* (70). No certain explanation for the proliferation-inducing effects of GPR55 overexpression is available so far. However, there are some data connecting these effects with the presence of LPI in experimental settings. Andradas et al. demonstrated that inhibition of cytosolic phospholipase A₂ (cPLA₂) activity with pyrrophenone in HEK293 cells blocks proliferation induced by GPR55 overexpression (70). Piñeiro et al. achieved the same effect after genetic ablation of cPLA₂. Importantly, cell proliferation decreased due to cPLA₂ silencing was recovered by addition of exogenous LPI (71) indicating that cancer cells generate mitogenic LPI through the action of cPLA₂. According to previously mentioned data concerning elevated levels of LPI in ascites and plasma from patients with ovarian cancer, the same proliferation induction strategy could be used, for instance, by human gynecological tumors.

CONCLUSIONS

Research conducted in past years has demonstrated that many important physiological and pathophysiological processes are regulated by LPC and LPI. LPC is identified as a ligand of GPR119 receptor expressed at high level by cells of Langerhans islets. The compound is proposed to be involved in carbohydrate metabolism modulation due to documented stimulation of insulin secretion and cellular glucose uptake. There are also some contradictory research leading to conclusion that LPC is either promoting or attenuating development of obesity and related metabolic disorders. Yet, it was described as a favorable means of weight gain promotion in case of animal husbandry. Apart from metabolism-related issues, LPC was found to promote wound healing, neuroregeneration processes and nervous system regulation, macular regeneration, and attenuation of autoimmune response. Molecular mechanisms of these activities remain poorly understood so far. However, there appeared several attempts to prove and explain the dependence of particular LPC activity on its species and chemical modifications.

LPI is defined as endogenous natural ligand for GPR55. The species is known as intracellular and cytoplasmic Ca²⁺ concentration regulator. LPI/GPR55 system was also demonstrated to be positively associated with obesity in human. Recent

Table 2. Update on recognized activities and mechanisms of action of LPC and LPI.

LPL	Non-receptor mediated effects	Influenced receptor	Receptor distribution	Identified LPL mediated G protein release	Main signaling pathways	Receptor mediated effects	References
LPC	<ul style="list-style-type: none"> • Counteracting neurodegeneration <ul style="list-style-type: none"> • Anti-inflammatory activity (polyunsaturated fatty acid residues) • Promotion of intestinal uptake of β-carotene and lutein (applicable in therapy of AMD and age-related diseases) • Reduction of aging process due to dietary uptake • Natural airway surfactant, medium for efficient airway gene transfer techniques • Synaptic function modulation due to regulation of the neuron membrane potential (activation of TREK and TRAAK K^+ channels) 	GPR4	Ubiquitous, mainly vascular endothelial cells, smooth muscle cells, lung, heart, kidney	$G_{\alpha_{16}}$?	$[Ca^{2+}]_{i}^{\uparrow}$, ERK \uparrow	Cell migration, angiogenesis	
		G2A	Inflammatory cells, lymphoid tissues, hematopoietic cells, keratinocytes	$G_{\alpha_{16}}$, $G_{\alpha_{11}}$, $G_{\alpha_{13}}$	$[Ca^{2+}]_{i}^{\uparrow}$, ERK \uparrow , p38 MAPK \uparrow , JNK \uparrow	Suppression of auto-inflammatory response, cell migration (especially LPC20:4/22:6, 17-hydroxy-LPC22:6, 15-hydroxy-LPC20:4/20:5), apoptosis	1–61
		GPR119	Major in pancreatic Langerhans islets, endocrine cells of the gut, brain	$G_{\alpha_{16}}$	AC \uparrow , PKA \uparrow	Regulation of insulin secretion from pancreatic β cells	
LPI	<ul style="list-style-type: none"> • Synaptic function modulation due to regulation of the neuron membrane potential (activation of TREK and TRAAK K^+ channels) 	GPR55 (weak interaction)	Central nervous system, adrenal glands, testis, spleen, gastrointestinal tract, breast adipose tissues, endothelium (i.e., human vein endothelial cells)	$G_{\alpha_{12}}$, $G_{\alpha_{13}}$	$[Ca^{2+}]_{i}^{\uparrow}$, ERK \uparrow , PLC \uparrow , rhoA \uparrow , cdc42 \uparrow , rac1 \uparrow	Regulation of nervous system and bone morphogenesis, cell migration	
		GPR55 (possibly also GPR119)	Endothelial cells, platelets, peripheral blood neutrophils, mouse fibroblasts and macrophages, rat brain cells, spleen, neutrophils, mast cells, DRG neurones, cancer cell lines including ones obtained from breast, brain, cervix, skin, pancreas, liver, ovaries, prostate, hematological tumors	$G_{\alpha_{13}}$	$[Ca^{2+}]_{i}^{\uparrow}$, ERK \uparrow , rhoA \uparrow , Akt \uparrow	Insulin release from pancreatic islets, artery contraction, regulation of osteoclasts number and function, regulation of peripheral sensory neuron function and pathological pain, regulation of the immune system response during inflammatory condition, modulation of proliferation, differentiation, motility and tumorigenesis in diverse cell types	1, 62–110

studies strongly suggest that LPI/GPR55 plays an important role in processes related to inflammatory and neuropathic pain. Moreover, it was shown that LPI may be involved in the immune system response during inflammatory condition, as well as modulation of osteoclast physiology. LPI is also emerging as a key modulator of proliferation, differentiation, motility and tumorigenesis in diverse cell types.

The past decade was rich in new findings on various biological activities as well as associated physiological and pathophysiological roles of LPC and LPI. Despite the fact, numerous questions still need to be answered and further studies are necessary.

Acknowledgment

This work was supported by grants from the National Science Centre (grant no. 2011/01/B/ST5/06383 and grant no. 2013/11/N/NZ5/00270).

REFERENCES

1. Grzelczyk A., Gendaszewska-Darmach E.: *Biochimie* 95, 667 (2013).
2. Rytczak P., Drzazga A., Gendaszewska-Darmach E., Okruszek A.: *Bioorg. Med. Chem. Lett.* 23, 6794 (2013).
3. Di Menna L., Molinaro G., Di Nuzzo L., Riozzi B., Zappulla C., Pozzilli C., Turrini R. et al.: *Pharmacol. Res.* 67, 1 (2013).
4. Abramowski P., Otto B., Martin R.: *PLoS One* 9, e91970 (2014).
5. Gendaszewska-Darmach E.: *Acta Biochim. Pol.* 55, 227 (2008).
6. Benesch M.G., Ko Y.M., McMullen T.P., Brindley D.N.: *FEBS Lett.* (2014).
7. Grimm M.O., Grosgen S., Reimenschneider M., Tanila H., Grimm H.S., Hartmann T.: *J. Chromatogr A* 1218, 7713 (2011).
8. Riederer M., Ojala P.J., Hrzenjak A., Graier W.F., Malli R., Tritscher M., Hermansson M. et al.: *J. Lipid Res.* 51, 2957 (2010).
9. Jin M.C., Hung N.D., Yoo J.M., Kim M.R., Sok D.: *J. Lipid. Sci. Technol.* 114, 114 (2012).
10. Mamatha B.S., Baskaran. V.: *Nutrition* 27, 960 (2011).
11. Yea K., Kim J., Yoon J.H., Kwon T., Kim J.H., Lee H.J., Kim J.I. et al.: *J. Biol. Chem.* 284, 33833 (2009).
12. Overton H.A., Fyfe M.C.T., Reynet C.: *Br. J. Pharmacol.* 153, 76 (2008).
13. Soga T., Ohishi T., Matsui T., Saito T., Matsumoto M., Takasaki J., Matsumoto S. et al.: *Biochem. Biophys. Res. Commun.* 326, 744 (2005).
14. Oka S., Nahajima K., Yamashita A., Kishimoto S., Sugiura T.: *Biochem. Biophys. Res. Commun.* 362, 928 (2007).
15. Gendaszewska-Darmach E., Laska E., Rytczak P., Okruszek A.: *Bioorg. Med. Chem. Lett.* 22, 2698 (2012).
16. Ishikawa M., Maekawa K., Saito K., Senoo Y., Urata M., Murayama M., Tajima Y. et al.: *PLoS One* 9, 91806 (2014).
17. Schmitz G., Ruebsaamen K.: *Atherosclerosis* 208, 10 (2010).
18. Guo Z., Vikbjerg A.F., Xu X.: *Biotechnol. Adv.* 23, 203 (2005).
19. Lee J.Y., Min H.K., Moon M.H.: *Anal. Bioanal. Chem.* 400, 2953 (2011).
20. Akoi J., Taira Y., Takanezawa Y., Kishi Y., Hama K., Kishimoto T., Mizuno K. et al.: *J. Biol. Chem.* 277, 48737 (2002).
21. Murakami M., Taketomi Y., Miki Y., Sato H., Hirabayashi T., Yakamoto K.: *Prog. Lipid. Res.* 50, 152 (2011).
22. Xu Y.: *Biochim. Biophys. Acta.* 1582, 81 (2002).
23. Stock C., Schilling T., Schwab A., Eder C.: *J. Immunol.* 177, 8560 (2006).
24. Hasegawa H., Lei J., Matsumoto T., Onishi S., Suemori K., Yasukawa M.: *Biochem. Biophys. Res. Commun.* 415, 526 (2011).
25. Schilling T., Eder C.: *Cell. Immunol.* 256, 87 (2010).
26. Lucas A., Grynberg A., Lacour B., Goirand F.: *Metabolism* 57, 233 (2008).
27. Hung N.D., Sok D.E., Kim M.R.: *Inflamm. Res.* 61, 473 (2012).
28. Hung N.D., Kim M.R., Sok D.E.: *Br. J. Pharmacol.* 165, 1119 (2011).
29. Hung N.D., Kim M.R., Sok D.E.: *Prostaglandins Other Lipid Mediat.* 90, 105 (2009).
30. Meyer zu Heringdorf D., Jakobs K.H.: *Biochim. Biophys. Acta* 1768, 923 (2007).
31. Ben-Zeev G., Telias M., Nussinovitch I.: *Cell Calcium* 47, 514 (2010).
32. Eckels P., Gamboni-Robertson F., Banerjee A., Silliman C.C.: *Biochem. J.* 432, 35 (2010).
33. Matsuzaki K., Hanola T., Miyajima K., Mikura Y., Shimizu H., Toguchi H.: *Chem. Pharm. Bull.* 36, 4253, (1988).
34. Heerklotz H., Epanand R. M.: *Biophys. J.* 80, 271 (2001).
35. Kim Y.L., Im Y.J., Ha N.C., Im D.S.: *Prostaglandins Other Lipid Mediat.* 83, 130 (2007).

36. Rao S.P., Reiderer M., Leichleitner M., Hermansson M., Desoye G., Hallstrom S., Graier W.F., Frank S.: *PLoS One*. 8, 65155 (2013).
37. Overton H.A., Babbs A.J., Doel S.M., Fyfe M.C.T., Gardner L.S., Griffin G., Jackson H.C. et al.: *Cell Metabol.* 3, 167 (2006).
38. Amisten S., Salehi A., Rorsman P., Jones P.M., Persaud S.J.: *Pharmacol. Ther.* 139, 359 (2013).
39. Sakamoto Y., Inoue H., Kawakami S., Miyawaki K., Miyamoto T., Mizuta K., Itakura M.: *Biochem. Biophys. Res. Commun.* 351, 474 (2006).
40. Ahlkvist L., Brown K., Ahren B.: *Ahkvist. Endocr. Connect.* 2, 69 (2013).
41. Zhu X., Huang W., Qian H.: GPR119 Agonists: A Novel Strategy for Type 2 Diabetes Treatment, in *Diabetes Mellitus – Insights and Perspectives.*, Oguntibeju O. O. Ed., InTech, Rijeka 2013.
42. Lipina C., Rastedt W., Irving A.J., Hundal H.S.: *Bioessays* 34, 681 (2012).
43. Sena C.M., Bento C.F., Pereira P., Seiça R.: *EPMA J.* 326, 744 (2010).
44. Barber M.N., Risis S., Yang C., Meikle P.J., Staples M., Febbraio M.A., Bruce C.R.: *PLoS One* 7, 41456 (2012).
45. Wang-Sattler R., Yu Z, Herder C, Messias AC, Floegel A, He Y, Heim K, et al.: *Mol. Syst. Biol.* 8, 615 (2012).
46. Esinger K., Liebisch G., Schmitz G., Aslanidis C., Krautbauer S., Buechler C.: *Int. J. Mol. Sci.* 15, 2991 (2014).
47. Li F., Jiang C., Larsen M. C., Bushkofsky J., Krausz K. W., Wang T., Jefcoate C. R., Gonzalez F. J.: *J. Prot. Res.* (2014).
48. Odori S., Hosoda K., Tomita T., Fujikura J., Kusakabe T., Kawaguchi Y., Doi R. et al.: *Metabolism* 62, 70 (2013).
49. Romero-Zerbo S.Y., Rafacho A., Diaz-Arteaga A., Suarez J., Quesada I., Imbernon M., Ross R.A. et al.: *J. Endocrinol.* 211, 177 (2011).
50. McKillop A.M., Moran B.M., Abdel-Wahab Y.H., Flatt P.R.: *Br. J. Pharmacol.* 170, 978 (2013).
51. Godlewski G., Offertaler L., Wagner J.A., Kunos G.: *Prostaglandins Other Lipid Mediat.* 89, 105 (2009).
52. Kotake-Nara E., Nagao A.: *Biosci. Biotechnol. Biochem.* 76, 875 (2012).
53. Rodriguez-Navarro J.A., Kaushik S., Koga H., Dall'Armi C., Shui G., Wenk M.R., Di Paolo G., Cuervo A.M.: *Proc. Natl. Acad. Sci. USA* 109, 705 (2012).
54. Abdolahi A., Georas S.N., Brenna J.T., Cai X., Thevenet-Morrison K., Phipps R.P., Lawrence P. et al.: *Prostaglandins Leukot. Essent. Fatty Acids* 90, 61 (2014).
55. Oresic M., Seppanen-Laakso T., Sun D., Tang J., Therman S., Viehman R., Mutsonen U. et al.: *Genome Med.* 4, 1 (2012).
56. Zhang B., Haitao L., Zhao D., Guo Y., Barri A.: *Anim. Feed Sci. Technol.* 163, 177 (2011).
57. Raju M.V., Rao S.V., Chakrabarti P.P., Rao B.V., Panda A.K., Devi B., Sujatha V. et al.: *Br. Poult. Sci.* 52, 769 (2011).
58. Nakano T., Inoue I., Alpers D.H., Akiba Y., Katayama S., Shinozaki R., Kaunitz J.D. et al.: *Am. J. Physiol. Gastrointest. Liver Physiol.* 297, 207 (2009).
59. Kogure K., Nakashima S., Tsuchie A., Tokumura A., Fukuzawa K.: *Chem. Phys. Lipids*.126, 29 (2003).
60. Cmielewski P., Anson D.S., Parson D.W.: *Respir. Res.* 11, 84 (2010).
61. Kitsioulis E., Nakos G., Lekka M. E.: *Biochim. Biophys. Acta* 1792, 941 (2009).
62. Bondarenko A., Waldeck-Weiermair M., Naghdi S., Poteser M., Malli R., Graier W.F.: *Br. J. Pharmacol.* 161, 308 (2010).
63. Billah M.M., Lapetina E.G.: *J. Biol. Chem.* 257, 5196 (1982).
64. Smith D.M., Waite M.: *J. Leukoc. Biol.* 52, 670 (1992).
65. Xiao Y., Chen Y., Kennedy A.W., Belinson J., Xu Y.: *Ann. N.Y. Acad. Sci.* 905, 242 (2000).
66. Xiao Y.J., Schwartz B., Washington M., Kennedy A., Webster K., Belinson J., Xu Y.: *Anal. Biochem.* 290, 302 (2001).
67. Xu Y., Xiao Y.J., Baudhuin L.M., Schwartz B.M.: *J. Soc. Gynecol. Investig.* 8, 1 (2001).
68. Ford L.A., Roelofs A.J., Anavi-Goffer S., Mowat L., Simpson D.G., Irving A.J., Rogers M.J. et al.: *Br. J. Pharmacol.* 160, 762 (2010).
69. Oka S., Ota R., Shima M., Yamashita A., Sugiura T.: *Biochem. Biophys. Res. Commun.* 395, 232 (2010).
70. Andradas C., Caffarel M.M., Perez-Gomez E., Salazar M., Lorente M., Velasco G., Guzman M., Sanchez C.: *Oncogene* 30, 245 (2011).
71. Piñeiro R., Maffucci T., Falasca M.: *Oncogene* 30, 142 (2011) .
72. Cantarella G., Scollo M., Lempereur L., Saccani-Jotti G., Basile F., Bernardini R.: *Biochem. Pharmacol.* 82, 380 (2011).
73. Hong S.L., Deykin D.: *J. Biol. Chem.* 256, 5215 (1981).

74. Zoeller R.A., Wightman P.D., Anderson M.S., Raetz C.R.: *J. Biol. Chem.* 262, 17212 (1987) .
75. Oka S., Toshida T., Maruyama K., Nakajima K., Yamashita A., Sugiura T.: *J. Biochem.* 145, 13 (2009).
76. Yamashita A., Oka S., Tanikawa T., Hayashi Y., Nemoto-Sasaki Y., Sugiura T.: *Prostaglandins Other Lipid Mediat.* 107, 103 (2013).
77. Murase S., Okuyama H.: *J. Biol. Chem.* 260, 262 (1985).
78. Tsutsumi T., Kobayashi T., Ueda H., Yamauchi E., Watanabe S., Okuyama H.: *Neurochem. Res.* 19, 399 (1994).
79. Umezū-Goto M., Kishi Y., Taira A., Hama K., Dohmae N., Takio K., Yamori T. et al.: *J. Cell Biol.* 1508, 227 (2002).
80. Metz S.A.: *Biochem. Biophys. Res. Commun.* 138, 720 (1986).
81. Metz S.A.: *J. Pharmacol. Exp. Ther.* 238, 819 (1986).
82. Oka S., Nakajima K., Yamashita A., Kishimoto S., Sugiura T.: *Biochem. Biophys. Res. Commun.* 362, 928 (2007).
83. Moreno-Navarrete J.M., Catalan V., Whyte L.: *Diabetes* 61, 281 (2012).
84. Ross R.A.: *Trends Pharmacol. Sci.* 32, 265 (2011).
85. Monet M., Gkika D., Lehen'kyi V., Pourtier A., Abeele F.V., Bidaux G., Juvin V. et al.: *Biochim. Biophys. Acta* 1793, 528 (2009) .
86. Piñeiro R., Falasca M.: *Biochim. Biophys. Acta* 1821, 694 (2012) .
87. Andersson D.A., Nash M., Bevan S.: *J. Neurosci.* 27, 3347 (2007).
88. Vanden Abeele F., Zholos A., Bidaux G., Shuba Y., Thebault S., Beck B., Flourakis M. et al.: *J. Biol. Chem.* 281, 40174 (2006).
89. Lauckner J.E., Jensen J.B., Chen H.Y., Lu H.C., Hille B., Mackie K.: *Proc. Natl. Acad. Sci. USA* 105, 2699 (2008).
90. Henstridge C.M., Balenga N.A., Ford L.A., Ross R.A., Waldhoer M., Irving A.J.: *FASEB J.* 23, 183 (2009).
91. Balenga N.A., Aflaki E., Kargl J.: *Cell Res.* 21, 1452 (2011).
92. Whyte L.S., Ryberg E., Sims N.A., Ridge S.A., Mackie K., Greasley P.J.: *Proc. Natl. Acad. Sci. USA* 106, 16511 (2009).
93. Ryberg E., Larsson N., Sjogren S., Hjorth S., Hermansson N.O., Leonova J.: *Br. J. Pharmacol.* 152, 1092 (2007).
94. Sawzdargo M., Nguyen T., Lee D.K., Lynch K.R., Cheng R., Heng H.H., George S.R., O'Dowd B.F.: *Brain Res. Mol. Brain Res.* 64, 193 (1999).
95. Gangadharan V., Selvaraj D., Kurejova M., Njoo C., Gritsch S.: *Pain* 154, 2801 (2013).
96. Staton P.C., Hatcher J.P., Walker D.J.: *Pain* 139, 225 (2008).
97. Xu Y., Xiao Y.J., Baudhuin L.M., Schwartz B.M.: *J. Soc. Gynecol. Investig.* 8, 1 (2001).
98. Raftopoulou M., Hall A.: *Dev. Biol.* 265, 23 (2004).
99. Ridley A.J., Schwartz M.A., Burridge K., Firtel R.A., Ginsberg M.H., Borisy G.: *Science* 302, 1704 (2003).
100. Wheeler M.B., Seidel Jr. G.E.: *Gamete Res.* 22, 193 (1989).
101. Kohno M., Yokokawa K., Yasunari K., Minami M., Kano H., Hanehira T., Yoshikawa J.: *Circulation* 98, 353 (1998).
102. Murugesan G., Fox P.L.: *J. Clin. Invest.* 97, 2736 (1996).
103. Kume N., Cybulsky M.I., Gimbrone Jr M.A.: *J. Clin. Invest.* 90, 1138 (1992).
104. Alonso T., Santos E.: *Biochem. Biophys. Res. Commun.* 171, 14 (1990) .
105. Falasca M., Iurisci C., Carvelli A., Sacchetti A., Corda D.: *Oncogene* 16, 2357 (1998).
106. Eichholtz T., Jalink K., Fahrenfort I., Moolenaar W.H.: *Biochem. J.* 291, 677 (1993).
107. Labonte E.D., Pfluger P.T., Cash J.G., Kuhel D.G., Roja J.C., Magness D.P., Jandacek R.J. et al.: *FASEB J.* 24, 2516 (2010).
108. Labonte E.D., Kirby R.J., Schildmeyer N.M., Cannon A.M., Huggins K.W., Hui D.Y.: *Diabetes* 55, 935 (2006).
109. Kim H.J., Kim H.J., Noh S., Hur H.J., Sung M.J., Hwang J.T., Park J.H. et al.: *J. Proteome Res.* 10, 722 (2011).
110. Fox T.E., Bewley M.C., Unrath K.A., Pedersen, M.M., Anderson R.E., Jung D.Y., Jefferson L.S. et al.: *J. Lipid Res.* 52, 509 (2011).