Acyl Chain-Dependent Effect of Lysophosphatidylcholine on Endothelium-Dependent Vasorelaxation

Shailaja P. Rao1, Monika Riederer1,5, Margarete Lechleitner1, Martin Hermansson2, Gernot Desoye3, Seth Hallström4, Wolfgang F. Graier1, Sasa Frank1*

1 Institute of Molecular Biology and Biochemistry, Center of Molecular Medicine, Medical University Graz, Graz, Austria, 2 Department of Biochemistry, Institute of Biomedicine, University of Helsinki, Helsinki, Finland, 3 Clinic of Obstetrics and Gynecology, Medical University Graz, Graz, Austria, 4 Institute of Physiological Chemistry, Center of Physiological Medicine, Medical University Graz, Graz, Austria, 5 University of Applied Sciences, Biomedical Science, Graz, Austria

Abstract

Previously we identified palmitoyl-, oleoyl-, linoleoyl-, and arachidonoyl-lysocphatidylcholine (LPC 16:0, 18:1, 18:2 and 20:4) as the most prominent LPC species generated by endothelial lipase (EL). In the present study, we examined the impact of those LPC on acetylcholine (ACh)-induced vascular relaxation. All tested LPC attenuated ACh-induced relaxation, measured ex vivo, using mouse aortic rings and wire myography. The rank order of potency was as follows: 18:2>20:4>16:0>18:1. The attenuating effect of LPC 16:0 on relaxation was augmented by indomethacin-mediated cyclooxygenase (COX)-inhibition and CAY10441, a prostacyclin (PGI2)-receptor (IP) antagonist. Relaxation attenuated by LPC 20:4 and 18:2 was improved by indomethacin and SQ29548, a thromboxane A2 (TXA2)-receptor antagonist. The effect of LPC 20:4 could also be improved by TXA2- and PGI2-synthase inhibitors. As determined by EIA assays, the tested LPC promoted secretion of PGI2, TXA2, PGF2α, and PGE2, however, with markedly different potencies. LPC 16:0 was the most potent inducer of superoxide anion production by mouse aortic rings, followed by LPC 18:2, 20:4 and 18:1, respectively. The strong antioxidant tempol recovered relaxation impairment caused by LPC 18:2, 18:1 and 20:4, but not by LPC 16:0. The tested LPC attenuate ACh-induced relaxation through induction of proconstricting prostanooids and superoxide anions. The potency of attenuating relaxation and the relative contribution of underlying mechanisms are strongly related to LPC acyl-chain length and degree of saturation.

Introduction

Lysophosphatidylcholines (LPC) are bioactive phospholipids generated by various biological processes including: i) phospholipase A2 (PLA2)-catalysed cleavage of plasma membrane and lipoprotein phosphatidylcholine (PC) [1], ii) lecithin cholesterol acyltransferase (LCAT) activity in high density lipoproteins (HDL) [2], and iii) oxidation of low density lipoproteins (LDL) [3]. In contrast to exclusively saturated LPC species generated by aforementioned processes, both hepatic lipase (HL) and endothelial lipase (EL) generate in addition to LPC 16:0 unsaturated species 18:1, 18:2 and 20:4 by cleaving HDL-PC [4,5].

Under physiological conditions the plasma concentration of LPC is around 100–170 μM [6], with elevations under pathophysiological conditions up to millimolar concentrations in e.g. hyperlipidemic subjects [7]. LPC in plasma are distributed between albumin and other carrier proteins as well as lipoproteins [8,9]. Free LPC might occur locally during an excessive lipolysis and concomitant saturation of carrier proteins with lipolysis products. The interaction of free LPC with vascular endothelium, as found for LPC 16:0, results in altered endothelial function and impaired vascular reactivity [10,11].

The maintenance of normal vascular tone is largely dependent on the capability of vascular endothelium to maintain the fine balance between endothelium-derived relaxing factors (EDRF) and endothelium-derived contracting factors (EDCF). Upon stimulation with various agonists EDRF and EDCF released from vascular endothelial cells diffuse to underlying smooth muscle cells, where they act on specific receptors and cause relaxation or contraction [12]. While nitric oxide (NO) and endothelium-derived relaxants like PGI2, TXA2, causes contraction through activation of TP receptors [13]. However, under certain conditions, PGI2-like TXA2, causes contraction through activation of TXA2 receptors [14]. PGE2 promotes relaxation via PGE2 receptors subtypes EP2 and EP4, whereas by acting via EP1, EP3 and TP receptors it causes constriction [15,16]. Likewise, PGF2α and isoprostanes, cause contraction through activation of TP receptors on vascular smooth muscle cells [17,18]. Additionally, endothelium-derived ROS may act as potent EDCF either directly
by promoting depolarization of vascular smooth muscle [19] or indirectly by reducing NO bioavailability [20].

Previously we found that the capacity and underlying mechanisms of palmitoyl-LPC (16:0 LPC), oleyl-LPC (18:1 LPC), linoleoyl-LPC (18:2 LPC) and arachidonoyl-LPC (20:4 LPC) to modulate endothelial prostanoid production were remarkably different and related to the acyl-chain length as well as degree of saturation [21]. At present only the impact of 16:0 LPC on vascular reactivity has been investigated.

Here we tested the hypothesis of acyl chain dependency of LPC in altering vascular reactivity. To this end we compared the effects of LPC 18:1, 18:2 and 20:4 with LPC 16:0 on ACh-induced vasorelaxation in an ex vivo system using mouse aortic rings and myography. We found that the tested LPC attenuate ACh-induced relaxation through induction of proconstricting prostanoids and superoxide anions whereby the potency of attenuating relaxation and the relative contribution of underlying mechanisms are strongly related to LPC acyl-chain length and degree of saturation.

Materials and Methods

LPC

LPC 16:0, 18:1, 18:2 and 20:4 were purchased from Avanti Polar Lipids, Alabaster, AL or prepared as described [6]. LPC were dissolved in chloroform/methanol and stored at −20°C under argon atmosphere. Required amounts of LPC were dried under a stream of nitrogen or argon and re-dissolved in PBS (pH 7.4) before the experiment.

Mice and tissue preparation

Mice received care in accordance with the Austrian law on experimentation with laboratory animals, which is based on the U.S. National Institutes of Health guidelines. Male C57BL/6 mice (10–12 weeks old) provided by Himberg, Austria, were killed by cervical dislocation. The descending thoracic aorta was isolated and dissected free of adherent tissue.

Organ Chamber Experiments

Aortic rings approximately 2 mm in length were cut from descending thoracic aorta. The arterial rings were positioned in small wire myograph chambers (Danish MyoTechnology, Aarhus, Denmark), which contained physiological salt solution (PSS) (114 mM NaCl, 4.7 mM KCl, 0.8 mM KH₂PO₄, 1.2 mM MgCl₂, 2.5 mM CaCl₂, 25 mM NaHCO₃ and 11 mM D-glucose (pH 7.4) aerated with 5% CO₂/95% O₂ at 37°C). The myograph chambers were connected to force transducers for isometric myography. We found that LPC attenuate ACh-induced relaxations in an ex vivo system using mouse aortic rings and myography. We found that the tested LPC attenuate ACh-induced relaxation through induction of proconstricting prostanoids and superoxide anions whereby the potency of attenuating relaxation and the relative contribution of underlying mechanisms are strongly related to LPC acyl-chain length and degree of saturation.

Prostanoid measurement

Mouse aortic rings (approximately 2-mm in length) were incubated in 200 µl aerated PSS under cell culture conditions at 37°C for 1 h. Thereafter, buffer was replaced with fresh PSS supplemented with PSS (control) or LPC (10 µM) followed by further incubation under cell culture conditions at 37°C for 1 h. The buffers were flash frozen in liquid nitrogen for subsequent prostanoid quantification and rings were homogenized for protein quantification. Protein concentration was determined with the BCA protein assay kit (Novagen, Darmstadt, Germany). The concentrations of 6-Keto-PGF₁α, TXB₂, PGE₂ and PGF₂α were measured by corresponding correlate-EIA kits (Cayman, Ann Arbor, MI) according to the manufacturer’s protocol.

Superoxide anions measurement

Superoxide anions were measured as described [22] with some modifications. Mouse aortic rings were equilibrated in 100 µl PSS buffer containing 10 µM DETCA, an SOD-inhibitor and 10 µM lucigenin (Sigma) at 37°C for 30 minutes. LPC (10 µM) was added to the tubes immediately before measurements. The luminometer (Lumat LB9501, Berthold technologies, Germany) was set up to report arbitrary units of emitted light (RLU). Measurements were taken in triplicates every 10 seconds. In addition, blank measurements with and without aortic rings were collected in the same way to subtract background emission. The amounts of released superoxide anions (chemiluminescence units) were normalised to protein content of respective aortic rings. The RLU obtained in control incubations with PSS were set to 100% and the RLU obtained by LPC’s were expressed as percentage of the control.

Nitrite determination

Nitrite as an indicator of NO production was determined according to a previously described fluorometric HPLC method [23] utilizing the reaction of nitrite with 2,3-diaminonaphthalene (DAN). In brief, the nitrite levels were determined in the myography incubation buffers. Samples (300 µl) were taken and snap frozen in liquid nitrogen. After thawing 100 µl of the sample (incubation buffer) was incubated at 24°C with 10 µL of 316 µmol/L DAN (in 0.62 mol/L HCl) for 10 min, followed by addition of 10 µL of 2.8 mol/L NaOH. This reaction mixture was directly used for chromatographic separation (injection volume: 20 µL) of the formed 2, 3-naphthotriazole (NAT). Nitrite...
standards (range: 0–2 μmol/L) were derivatized accordingly. NAT was isocratically separated on a 5-μm ODS hypersil column (150×4.6 mm) guarded by a 5-μm ODS hypersil column (10×4.6 mm; Uniguard holder) with a 30 mmol/L sodium phosphate buffer (pH 7.5) containing 50% methanol (flow rate: 0.8 mL/min). Fluorescence was monitored at an excitation wavelength of 375 nm and an emission wavelength of 415 nm. The HPLC apparatus consisted of an L-2200 autosampler, L-2130 HTA pump and L-2480 fluorescence detector (VWR Hitachi, Tokyo, Japan). Detector signals were recorded with a personal computer. The EZchrom Elite (Scientific Software Inc., San Ramon, CA USA) was used for data requisition and analysis. The detection limit for nitrite was 10 pmol/mL.

Statistical Analysis

EC50 values (the ACh concentrations required to achieve 50% of maximal relaxation) are expressed as mean with 95% confidence intervals. Data are otherwise expressed as mean ± SEM. The significance of the difference between group means was analyzed by two-way analysis of variance and the Bonferroni-post test for samples. For prostanoid, nitrite and ROS measurements control and LPC treated aortic rings were compared by student’s T-test. Values of P<0.05 (*), P<0.01 (**), and P<0.001 (****) were taken as statistically significant. Statistical analysis was performed by Prism Version 4.0 (GraphPad Software, USA).

Results

LPC attenuate ACh-induced endothelium-dependent relaxation

All tested LPC attenuated aortic ring relaxation to cumulatively increasing concentrations of ACh with rank order of potency as follows: 18:2>20:4>16:0>18:1 (Fig. 1). EC50 values for LPC 18:2, 20:4, 16:0 and 18:1 were 334 nM (261–479), 298 nM (222–401), 214 nM (143–314) and 115 nM (86.2–155), respectively. The subsequent relaxation of the same rings to SNAP, following precontraction with NE, was not affected by either of the tested LPC (Fig. S1), indicating that responsiveness of aortic smooth muscle layers to NO was not impaired by prior exposure to LPC.
The role of COX and PGI2 in LPC-mediated attenuation of vascular relaxation

To examine whether COX-derived vasoconstricting prostanooids are responsible for the observed LPC-induced attenuation of relaxation, myography experiments were performed in the absence or presence of the non-selective COX inhibitor, indomethacin. In contrast to our expectation, indomethacin augmented the attenuating effect of LPC 16:0 on ACh-induced relaxation (Fig. 2A). Similar finding was obtained with CAY10441, a IP receptor antagonist (1 μM) for 30 minutes, followed by precontraction with NE and cumulative addition of ACh. Relaxation values were expressed as a percentage of the NE-induced contraction. Indomethacin improved relaxation attenuated by LPC 18:2 (C) and 20:4 (D). Relaxation attenuated by LPC 16:0 was exaggerated by indomethacin and CAY10441. Results are mean ± SEM of 20 rings for each case from 10 mice (A), 8 rings for each case from 3 mice (B) and 12 rings for each case from 6 mice (C,D).

doi:10.1371/journal.pone.0065155.g002

TP receptors are involved in LPC 18:2- and 20:4-mediated attenuation of vascular relaxation

Considering the well-established importance of TP receptors in mediating endothelium-dependent contractions [24,25], we examined whether SQ29548, a TP receptor antagonist could attenuate the inhibitory effect of LPC on vasorelaxation. SQ29548 markedly attenuated the inhibitory effect of LPC 18:2 (Fig. 3A) and significantly improved relaxation attenuated with LPC 20:4 (Fig. 3B), but had no significant impact on relaxation attenuated with LPC 16:0 or 18:1, respectively (not shown).

The role of TXA2 and PGI2 in LPC 18:2- and 20:4-mediated attenuation of vascular relaxation

Since TP receptors can be activated by both TXA2 and PGI2 [17], we tested the involvement of both prostanooids in LPC 18:2- and 20:4-induced attenuation of relaxation. Attenuated relaxation observed in the presence of LPC 20:4 was markedly improved upon inhibition of TXA2-synthase by furegrelate (Fig. 4A) as well as upon inhibition of PGI2-synthase by tranylcypromine (Fig. 4B). The co-application of both inhibitors resulted in further improvement of relaxation, however without complete restoration of relaxation, suggesting the involvement of additional 20:4 LPC-induced vasoconstricting factors. Neither of both inhibitors could recover relaxation impaired by LPC 18:2 (Fig. S3A, S3B).
Prostanoid release from LPC-treated aortic rings

To examine whether the production of prostanoids implicated in LPC-induced attenuation of relaxation (Figs. 1–4) was increased by LPC, we measured concentrations of prostanoids produced and secreted by aortic rings upon incubation with LPC. Compared with PSS-treated control incubations, LPC 20:4 was the most potent inducer of PGI2 production (measured as 6-Keto PGF1α, a stable degradation product of PGI2), followed by 18:2 and 16:0 (Fig. 5A). The effect of LPC 18:1 concerning prostanoids did not reach statistical significance (Fig. 5A). The release of TXB2 was significantly increased only with LPC 20:4 (Fig. 5B). While PGE2 production was significantly increased only upon incubation with LPC 20:4 (Fig. 5C), the levels of PGF2α were significantly increased with LPC 20:4 and 18:2, respectively (Fig. 5D).

LPC-induced oxidative stress contributes to LPC-induced attenuation of vascular relaxation

Since superoxide anions are established EDCF [17], we examined whether their production is triggered by LPC and whether they contribute to the observed LPC-induced impairment of relaxation. As shown in Figure 6A all LPC induced superoxide anion production in mouse aortic rings with the following order of potency: 16:0>18:2>20:4>18:1. The SOD mimetic tempol [26] improved relaxation impairment caused by LPC 18:1 (Fig. 6C), 18:2 (Fig. 6D) and 20:4 (Fig. 6E) but not that caused by LPC 16:0 (Fig. 6B).

Discussion

The present study investigated the effect of LPC 16:0, 18:1, 18:2 and 20:4, the most prominent LPC in human plasma [6], on endothelium-dependent relaxation in response to ACh. Previously, we identified these LPC as major hydrolysis products generated by the action of EL on HDL [4]. Considering the very high plasma levels under pathophysiological conditions (e.g., hyperlipidemic subjects) and their production by EL on the surface of vascular endothelial cells, these LPC might have a pronounced effect on endothelial function and vascular reactivity. Numerous studies have examined the effect of LPC on vascular reactivity. However, in these studies exclusively LPC 16:0 was used as a model LPC. To the best of our knowledge the present study is the first one to address the effect of unsaturated LPC 18:1, 18:2 and 20:4 on ACh-induced relaxation and to compare the effect with the saturated LPC 16:0.

We found that all LPC caused a pronounced attenuation of endothelium-dependent relaxation (EDR) to ACh with remarkable acyl-chain dependent differences regarding the potency and underlying mechanisms. LPC did not alter the SNP-induced relaxation (Fig. S1). This demonstrates that LPC are not toxic to smooth muscle cells and that the observed LPC-mediated attenuation of relaxation is an endothelium-dependent effect. Neither of the tested LPC modified the contractile response to NE, nor did they induce contraction when co-applied with the eNOS inhibitor L-NNA (not shown).

L-NNA completely inhibited, whereas indomethacin had no effect on ACh-induced relaxation of mouse aortic rings (Fig. S4A, S4B). Thus, NO seems to be the major mediator of ACh-induced relaxation in our experimental model. This is in line with the inability of ACh to alter vascular tone in aortic rings from eNOS deficient mice [27]. Accordingly, the observed attenuation of relaxation caused by LPC may reflect increased production and activity of EDCF, with concomitant counteraction of ACh-induced NO-mediated relaxation. This is similar to the inability of endothelial NO to curtail the effect of EDCF observed in arteries of aging and diseased (essential hypertension, diabetes) animals and humans [28–30]. In spontaneous hypertensive rats (SHR) and in essential hypertensive patients, impaired vasodilation was almost normalized by the COX-inhibitor indomethacin, indicating that COX-derived vasoconstrictors are key EDCF responsible for impaired endothelial function and blunted vasorelaxation [30].

Improvement of relaxation attenuated by LPC 18:2 and 20:4 upon inhibition of both COX (Fig. 2C, 2D) and TP receptors (Fig. 3A, 3B), indicated the involvement of COX-derived EDCF, which are capable of inducing contraction by acting via TP receptors [17,30]. These receptors are highly expressed in mouse aortic smooth muscle cells [31]. Both PGI2 and TXA2 were markedly induced by LPC 20:4 (Fig. 5A, 5B). These prostanoids are capable of activating TP receptors [17] and may, hence, have a major contribution to the LPC 20:4-effect (Fig. 4A–C). A recent study clearly showed that in mouse aorta both exogenously applied PGI2 (0.03 μM) as well as endogenous, ACh-induced PGI2 potently induced vasoconstriction by acting on TP receptor [32]. In that study, the concentration of 6-keto PGF1α upon ACh-stimulation was 2 pg/μg tissue, which is 20 times less than what we observed upon exposure of rings to 20:4 LPC (Fig. 5A).
Accordingly, 40 pg/µg of 6-keto PGF1α in 20:4 LPC-treated rings strongly argues for a PG12-induced vasoconstriction via TP receptors in our experimental model. Additionally, the decreased ability of IP receptors to promote relaxation, as found in vascular smooth muscles of SHR [30], and/or markedly higher levels of TP compared with IP receptors in mouse aortic smooth muscle cells [31], might facilitate vasoconstriction in 20:4 LPC-treated rings, despite increased PG12 production. Future experiments should reveal whether responsiveness of IP receptors to PG12 or its stable analogue, iloprost, is altered by LPC.

Neither TXA2 nor PG12 were involved in LPC 18:2-induced relaxation attenuation (Fig. S3A, S3B). However, the robust counteracting effect of the TP receptor antagonist on the LPC 18:2-induced relaxation attenuation (Fig. 3A) strongly suggests the existence and action of some LPC 18:2-induced TP-receptor agonists. Because LPC 18:2 induced PGF2α (Fig. 5D) which can

Figure 4. Inhibition of TXA2- and PG12- synthase improves relaxation attenuated by LPC 20:4. The rings were preincubated without (no LPC) or with LPC 20:4 in the absence or presence of 10 µM furegrelate, a TXA2 synthase inhibitor (A) or 10 µM tranylcypromine, a PG12 synthase inhibitor (B) or a combination of both (C) for 30 minutes, followed by precontraction with NE and cumulative addition of ACh. Results are mean ± SEM of 12 rings from 6 mice. *P<0.05, **P<0.01. doi:10.1371/journal.pone.0065155.g004
activate TP receptors [17], the LPC 18:2-induced attenuation of relaxation might at least in part be due to the EDCF-activity of induced PGF2α. Furthermore, isoprostanes such as 8-epi-PGF2α formed non-enzymatically by ROS induced peroxidation of cell membrane polyunsaturated fatty acids, as well as PGH2, a direct product of COX, might by acting via TP receptors, contribute to observed attenuating effect of LPC 18:2 on relaxation. Likewise, PGE2 and PGF2α[24] both markedly increased with LPC 20:4 (Fig. 5C, 5D) might contribute by acting via the TP receptors, to the relaxation attenuation induced by LPC 20:4. Due to the negligible expression of PGE2 receptors EP1 and EP3 [receptors associated with contraction] in mouse aortic smooth muscle cells, their contribution to contraction seems unlikely [27].

Previous studies found contradictory effects of LPC 16:0 on vasorelaxation, most probably attributable to vascular bed- and species- specific differences in tissue responsiveness to LPC [33–36]. In our experimental system LPC 16:0 increased the nitrite levels (indicative of NO levels) in organ bath of aortic rings exposed to ACh (Fig. S5), arguing against decreased NO as a cause of relaxation impairment induced by this LPC. Importantly, SNP-induced relaxation was not altered by the presence of LPC 16:0, indicating that responsiveness of smooth muscle cells to NO was not impaired by this LPC (Fig. S1A).

We found that in contrast to the tested unsaturated LPC species, the rate of ACh-induced relaxation in the presence of LPC 16:0 is the sum of 16:0-LPC-induced attenuation of relaxation (by a so far unknown mechanism) and promotion of relaxation by 16:0-LPC-induced PGI2 (Figs. 2B and 5A). By contrast, a similar induction of PGI2 by LPC 18:2 (Fig. 5A) failed to promote relaxation. This difference between LPC 16:0 and 18:2 is not clear, but one can speculate it might be due to the fact that PGI2 is the only prostanoid upregulated by LPC 16:0, whereas the action of LPC 18:2-induced PGI2 might be disturbed by concomitantly upregulated PGF2α or by detrimental effect of LPC 18:2 on IP receptor functionality.

Considering the short exposure of aortic rings to LPC (45 min) in our experiments, the observed augmenting effect of LPC 16:0 on prostanoid production may not reflect upregulation of
underlying enzymes such as COX-2 or respective prostanoid synthases. Indeed, in our recent study, the LPC-induced COX-2 protein upregulation in endothelial cells was detectable not earlier than after 3 h of incubation with LPC [37]. Hence, in line with our previous findings [21] the observed increase in prostanoid production upon exposure of aortic rings to LPC is rather a consequence of an acute effect of LPC, namely LPC-induced increase in cytosolic calcium concentration with concomitant activation of phospholipase-mediated release of arachidonic acid from membrane phospholipids. Because in contrast to LPC 16:0, 18:1 and 18:2, LPC 20:4 not only induces arachidonic acid release, but also provides its own arachidonic acid to COX [21], 20:4 LPC elicited the highest prostanoid production in aortic rings (Fig. 5A).

Besides vasoconstricting prostanoids, ROS are established EDCF [17]. In contrast to LPC 18:1, 18:2 and 20:4, LPC 16:0-induced attenuation of relaxation could not be improved by tempol, a SOD mimetic [26] (Fig. 6). Similarly, LPC 16:0 promoted ROS production in rat aortic rings, but MnCl₂, another SOD mimetic, failed to restore the impaired relaxation [36]. As found for tempol, a combination of 10 μM DETCA (SOD inhibitor) and 100 μM TIRON (ROS scavenger) failed to improve relaxation attenuated by LPC 16:0 (Fig. S6). The most prominent tempol-mediated improvement of relaxation was observed with LPC 18:1, most likely due to the fact that only increased ROS and not concomitantly increased vasoconstricting prostanoids underlie the LPC 18:1-induced relaxation attenuation.

Since LPC used in the present study were prepared by PLA₂-mediated cleavage of di-16:0-, -18:1-, -18:2- and -20:4-PC [6], applied LPCs are sn-1-acyl sn-2-lyso LPC. This is in a good accordance with situation in vivo where sn-1-lyso sn-2-acyl LPC generated by sn-1 phospholipases (such as EL or HL) give rise to sn-1-acyl isomers due to a rapid migration of acyl chains (in aqueous medium at neutral pH at 37°C) from the sn-2 to the deacylated sn-1 position to give a more stable intermediate [38]. Whether sn-1 and sn-2 LPC isomers differ in their biological activities regarding modulation of endothelial function and vascular reactivity remains to be determined.

Based on our results, LPC 16:0, 18:1, 18:2 and 20:4 emerge as important triggers of endothelial dysfunction. The major players responsible for the blunted endothelium-dependent relaxation in aged vessels and in various pathologies (essential hypertension, diabetes and atherosclerosis) are vasoconstricting prostanoids and ROS [17,28]. The fact that the studied LPC promote the production of these established EDCF strongly argues for the role of LPC 16:0, 18:1, 18:2 and 20:4 as important contributors to endothelial dysfunction in aging and aforementioned pathologies.

Future experiments should reveal the relationship between plasma levels of those LPC and the incidence and degree of endothelial dysfunction in humans and animal models of hypertension.

Supporting Information

Figure S1 SNP-induced relaxation is not affected by prior exposure of rings to LPC. The rings were preincubated without (no LPC) or with 10 μM LPC 16:0 (A), 18:1 (B), 18:2 (C) or 20:4 (D) for 30 minutes, followed by precontraction with NE and cumulative addition of ACh. Results for each condition are mean ± SEM of 12 rings from 6 mice. *P<0.05, **P<0.01.

doi:10.1371/journal.pone.0065155.g006
with PSS. Thereafter, the rings were precontracted with NE, followed by cumulative addition of SNP (0.1 nM to 30 nM). Relaxation values were expressed as a percentage of the NE-induced contraction. Results of each experimental condition are mean ± SEM of 8 rings for each case from 4 mice. (TIF)

Figure S5 LPC 16:0 induces nitrite levels released from aortic rings exposed to Ach. The rings were precontracted with NE and relaxed by cumulative addition of Ach. After wash-out of Ach, the same rings were preincubated with 10 μM LPC 16:0 for 30 minutes followed by a new contraction-relaxation cycle. The nitrite levels were determined in incubation buffers after the first (control) and the second contraction-relaxation cycle (LPC 16:0). Results are mean ± SEM of 8 rings for each condition from 4 mice. When LPC was omitted the nitrite levels released from rings were similar in the first and the second contraction-relaxation cycle (not shown). (TIF)

Figure S6 Combination of DETCA (SOD inhibitor) and TIRON (superoxide ion scavenger) fail to counteract LPC 16:0-induced attenuation of relaxation. The rings were preincubated without (no LPC) or with 10 μM LPC 16:0 in the absence or presence of DETCA (10 μM) and TIRON (100 μM) for 30 minutes, followed by preconstriction with NE and cumulative addition of Ach. Results for each condition are mean ± SEM of 8 rings from 4 mice. (TIF)

Acknowledgments

We thank Isabella Hindler for help with the care of the mice.

Author Contributions

Conceived and designed the experiments: MR SF. Performed the experiments: SPR MR ML MH SH. Analyzed the data: SPR MR SH WFG SF. Contributed reagents/materials/analysis tools: GD WFG. Wrote the paper: SF GD WFG.

References

1. Sato H, Kato R, Isogai Y, Saka G, Ohtsuki M, et al. (2008) Analyses of group III secreted phospholipase A2 transgenic mice reveal potential participation of this enzyme in plasma lipoprotein modification, macrophage foam cell formation, and atherosclerosis. Journal of Biological Chemistry 283: 33483–33497.

2. Rouset X, Vaisman B, Amar M, Sethi AA, Remaley AT (2009) Lecithin: cholesterol acyltransferase—from biochemistry to role in cardiovascular disease. Current Opinion in Endocrinology, Diabetes & Obesity 16: 163–171.

3. Parthasarathy S, Steinbrecher UP, Barnett J, Witzum JL, Steinberg D (1985) Essential role of phospholipase A2 activity in endothelial cell-induced modification of low density lipoprotein. Proc Natl Acad Sci U S A 82: 3000–3004.

4. Gauster M, Rechberger G, Sovic A, Hofl G, Steyer E, et al. (2005) Endothelial lipase releases saturated and unsaturated fatty acids of high density lipoprotein phosphatidylcholine. J Lipid Res 46: 1517–1525.

5. Santamarina-Feo J, Gonzalez-Navarro H, Freeman L, Wagner E, Nong Z (2004) Hepatic lipase, lipoprotein metabolism, and atherogenesis. Arterioscler Thromb Vasc Biol 24: 1750–1754.

6. Ogala PJ, Hirvonen TE, Hermansson M, Somerharju P, Parkkinen J (2007) Acyl chain-dependent effect of lysophosphatidylcholine on human neutrophils. J Leukoc Biol 82: 1501–1509.

7. Chen L, Lang B, Froese DE, Liu S, Wong JT, et al. (1997) Oxidative modification of low density lipoprotein in normal and hyperlipidemic patients: effect of lysophosphatidylcholine composition on vascular relaxation. J Lipid Res 38: 546–553.

8. Croset M, Brossard N, Polette A, Lagarde M (2000) Characterization of plasma unesterified lysophosphatidylincholines in human and rat. Biochemical Journal 345 Pt 1: 61–67.

9. Ogala PJ, Hermansson M, Tolvanen M, Polvonen K, Hirvonen T, et al. (2006) Identification of alpha-1 acid glycoprotein as a lysophospholipid binding protein: a complementary role to albumin in the scavenging of lysophosphatidylcholine. Biochemistry 45: 14021–14031.

10. Zhang R, Bai N, So J, Laher I, MacLeod KM, et al. (2009) The ischemic metabolite lysophosphatidylcholine increases rat coronary arterial tone by endothelium-dependent mechanism. J Mol Cell Cardiol 47: 112–120.

11. Froese DE, McMaster J, Man RY, Choy PC, Kroeger EA (1999) Inhibition of endothelium-dependent vascular relaxation by lysophosphatidylcholine: impact of lysophosphatidylcholine on mechanisms involving endothelium-derived nitric oxide and endothelin derived hyperpolarizing factor. Mol Cell Biochem 197: 1–6.

12. Furchgott RF, Vanhoutte PM (1989) Endothelium-derived relaxing and contracting factors. FASEB J 3: 800–807.

13. Wong MS, Vanhoutte PM (2010) COX-mediated endothelium-dependent contractions: from the past to recent discoveries. Acta Pharmacol Sin 31: 1095–1102.

14. Williams SP, Dorn GW Jr, Rapoport RM (1994) Prostaglandin E2 mediates contraction and relaxation of vascular smooth muscle. Am J Physiol 267: H796–H803.

15. Coleman RA, Smith WL, Narumiya S (1994) International Union of Pharmacology classification of prostaglandin receptors: properties, distribution, and structure of the receptors and their subtypes. Pharmacol Rev 46: 205–229.

16. Gluais P, Payaut J, Badier-Commander C, Verbeuren T, Vanhoucke PM, et al. (2006) In SHR aorta, calcium ionophore A-23187 releases prostacyclin and thromboxane A2 as endothelium-derived contracting factors. Am J Physiol Heart Circ Physiol 291: H2255–H2264.

17. Tang EH, Vanhoucke PM (2009) Prostanoids and reactive oxygen species: team players in endothelium-dependent contractions. Pharmacol Ther 122: 140–149.

18. Gluais P, Louchart M, Morrow JD, Vanhoucke PM, Feletou M (2005) Acetylsalicylic acid-induced endothelium-dependent contractions in the SHR aorta: the Janus face of prostacyclin. Br J Pharmacol 146: 434–445.

19. Tang XD, Garcia ML, Heinemann SH, Hoshi T (2004) Reactive oxygen species impair Sk1 BK channel function by altering cysteine-mediated calcium sensing. Nat Struct Mol Biol 11: 171–178.

20. Rahbari GM, Vanhoucke PM (1986) Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. Am J Pharmacol 250: H822–H827.

21. Riederer M, Ogala PJ, Hrzenjak A, Graier WF, Malli R, et al. (2010) Acyl chain-dependent effect of lysophosphatidylcholine on endothelial prostacyclin production. J Lipid Res 51: 2957–2966.

22. Dikalov S, Griendling KK, Harrison DG (2007) Measurement of reactive oxygen species in cardiovascular studies. Hypertension 49: 717–727.

23. Li H, Meininger CJ, Wu G (2000) Rapid determination of nitrite by reversed-phase high-performance liquid chromatography with fluorescence detection. J Chromatogr B Biomed Sci Appl 746: 199–207.

24. Tang EH, Jensen BL, Skott O, Leung GP, Feletou M, et al. (2008) The role of prostaglandin E and thromboxane-prostanoid receptors in the response to...
prostaglandin E2 in the aorta of Wistar Kyoto rats and spontaneously hypertensive rats. Cardiovasc Res 78: 130–138.

25. Zhou Y, Varadharaj S, Zhao X, Parinandi N, Flavahan NA, et al. (2005) Acetylcholine causes endothelium-dependent contraction of mouse arteries. Am J Physiol Heart Circ Physiol 289: H1027–1032.

26. Simonsen U, Christensen FH, Baus NH (2009) The effect of tempol on endothelium-dependent vasodilatation and blood pressure. Pharmacol Ther 122: 109–124.

27. Hristovska AM, Rasmussen LE, Hansen PB, Nielsen SS, Nusing RM, et al. (2007) Prostaglandin E2 induces vascular relaxation by E-prostanoid 4 receptor-mediated activation of endothelial nitric oxide synthase. Hypertension 50: 525–530.

28. Vanhoutte PM, Shimokawa H, Tang EH, Feletou M (2009) Endothelial dysfunction and vascular disease. Acta Physiol (Oxf) 196: 193–222.

29. Tang EH, Ku DD, Tipoe GL, Feletou M, Man RY, et al. (2005) Endothelium-dependent contractions occur in the aorta of wild-type and COX2-/- knockout but not COX1-/- knockout mice. J Cardiovasc Pharmacol 46: 761–765.

30. Feletou M, Verbeuren TJ, Vanhoutte PM (2009) Endothelium-dependent contractions in SHR: a tale of prostanoid TP and IP receptors. Br J Pharmacol 156: 563–574.

31. Fujino T, Yuki K, Yamada T, Hara A, Takahata O, et al. (2002) Effects of the prostanoids on the proliferation or hypertrophy of cultured murine aortic smooth muscle cells. Br J Pharmacol 136: 530–539.

32. Liu B, Luo W, Zhang Y, Li H, Zhu N, et al. (2012) Involvement of cyclooxygenase-1-mediated prostacyclin synthesis in the vasoconstrictor activity evoked by ACh in mouse arteries. Exp Physiol 97: 277–289.

33. Wolf A, Saito T, Dudek R, Bing RJ (1991) The effect of lysophosphatidylcholine on coronary and renal circulation in the rabbit. Lipids 26: 223–226.

34. Menon NK, Bing RJ (1991) Nitroarginine does not inhibit lysophosphatidylcholine (LPC)-induced vascular relaxation and accumulation of cyclic GMP. Proc Soc Exp Biol Med 196: 461–463.

35. Dudek R, Conforto A, Bing RJ (1993) Lysophosphatidylcholine-induced vascular relaxation and production of cGMP are mediated by endothelium-derived relaxing factor. Proc Soc Exp Biol Med 203: 474–479.

36. Yuong TD, de Kimpe S, de Roos R, Rabelink TJ, Koomans HA, et al. (2001) Albumin restores lysophosphatidylcholine-induced inhibition of vasodilation in rat aorta. Kidney Int 60: 1088–1096.

37. Brkic L, Riederer M, Graier WF, Malli R, Frank S (2012) Acyl chain-dependent effect of lysophosphatidylcholine on cyclooxygenase (COX)-2 expression in endothelial cells. Atherosclerosis 224: 548–554.

38. Phulikhit A, Dennis EA (1992) Acyl and phosphoryl migration in lysophospholipids: importance in phospholipid synthesis and phospholipase specificity. Biochemistry 21: 1743–1750.